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Section B.—Biological Sciences.

*Experimental Researches on Vegetable Assimilation and Respiration.** V.—*A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves.*

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(Communicated by Dr. F. F. Blackman, F.R.S. Received June 11,—Read June 24, 1909.)

CONTENTS.

Section I.—Introduction.

The trustworthiness of Sachs' well-known dry-weight method† for measuring the rate of accumulation and translocation of the products of

* Earlier contributions to this series of investigations carried out under the general direction of Dr. F. F. Blackman at Cambridge are the following: I and II, Blackman, 'Phil. Trans.,' B, 1895; III, Matthaei, 'Phil. Trans.,' B, 1904; and IV, Blackman and Matthaei, 'Roy. Soc. Proc.,' B, 1905.

† Sachs, Julius von, "Ein Beitrag zur Kenntniss der Ernährungsthätigkeit der Blätter," 'Arbeit. d. Bot. Inst. in Würzburg,' III, 1883, p. 19.

photosynthesis in leaves was called in question in 1905 by Brown and Escombe. In their paper "On the Physiological Processes of Green Leaves,"* they published an account of four experiments in which they had determined for the same individual leaves both the increase of dry weight, by Sachs' method, and the amount of carbon dioxide actually absorbed, by their own method. They sum up the results by saying: "If we take the mean of all four experiments we find that the Sachs method gives an estimate of the assimilation rate between two and three times greater than that deduced from the intake of carbon dioxide."†

In attempting to explain this discrepancy they made some determinations of the degree of symmetry existing between opposite sides of various leaves, and of the amount by which half-leaves changed in area under experimental conditions. They concluded from these that the errors to which the method is liable are of the same order of magnitude as the quantities to be measured, and that therefore "the Sachs method cannot be trusted for anything like exact quantitative estimation of the photosynthetic work which is going on in an assimilating leaf. As ordinarily applied, its general tendency is to give far too high an estimate of the rate of assimilation."‡

Since Sachs' method is the only one available for measuring the total photosynthetic activity of leaves under *natural conditions*, it is of the utmost importance that it should not lightly be abandoned. Its loss would be felt in pure physiology, and perhaps still more in ecology. I therefore undertook, at Dr. F. F. Blackman's suggestion, a detailed investigation of the Sachs method, in order to discover whether it was completely untrustworthy, or whether its weaknesses could be overcome.

Since the present paper is concerned principally with the results of this examination of the method, much is included that is technical and of importance only to those who wish to use the method;§ but the nature of the changes occurring in the leaf lamina during insolation (Section III) and the phenomena of shrinkage and expansion in area which follow variations in the intensity of illumination (Section V) are subjects of more general interest. Some applications of the method will be described in a later paper.

The work was carried out in the Cambridge University Botany School during my tenure of the Cambridge University Frank Smart Studentship. My thanks are due to Dr. F. F. Blackman for his kind interest in the work throughout its course and for many helpful suggestions.

* 'Roy. Soc. Proc.,' B, vol. 76, 1905, p. 29.

† *Loc. cit.*, p. 58.

‡ *Loc. cit.*, p. 61.

§ The most technical portions are in smaller type.

Section II.—On the Nature of the Errors to which the Half-leaf Dry-Weight Method is Liable.

The Sachs method consists essentially in the comparison of the dry weight per unit area of one half of a leaf (the control half-leaf) at the beginning of an experiment, with that of the other (the experimental half-leaf) after a certain number of hours of assimilation. The difference is taken to be the weight of the products which have been accumulated by the leaf during that time per unit of area.

Underlying the method are two assumptions, (1) that the symmetry is perfect, *i.e.* that the two halves of every leaf used possess appreciably the same dry weight per unit area, and (2) that no change takes place in the experimental half leaf to alter the original dry weight per unit area except the accumulation of the products of photosynthesis which it is the object of the experiment to measure.

The few experiments of Brown and Escombe clearly prove these assumptions to be unjustifiable, as leaves are far from symmetrical, and leaf area may change under experimental conditions. Further, to help explain the recorded excessive gain of dry weight during assimilation, they suggest a possible increase in the retention of water by colloids of the insolated leaf.*

If increase of dry weight were partly due to such an indeterminable fixation of water, the increase could not be used as a measure of assimilation, and the whole procedure would fall to the ground. This fear will be shown in Section III to be without foundation.

Catalpa bignonioides, used by Brown and Escombe in their test of the dry-weight method, happens to have been an unfortunate choice, since its leaves are particularly unsymmetrical in respect of dry weight. Other leaves show a much closer agreement between the two halves. The degree of asymmetry of a number of species is dealt with in Section IV.

Change of area has proved to be of extreme importance, as it is to errors from this source that the observed tendency to high results is due. Section V is devoted to this question.

In later sections of the paper minor errors of technique are carefully considered and estimated. Errors in the measurement of area, errors in weighing due to incomplete drying of the hygroscopic leaf material, etc., become of real importance, because their effects are cumulative in the resulting error in the observed gain of dry weight.

The following is a formal analysis of the various sources of error. Of those classed as errors of interpretation, that due to the varying composition

* *Loc. cit.* p. 59.

of the increase is briefly dealt with in Section III; the others are outside the scope of the present paper :—

Summary of Sources of Error affecting the Half-leaf Dry Weight Method.

i. Errors introduced in any single case in estimating the dry weight of the unit of area of leaf lamina :
- *a.* In measuring or determining the area, by the various methods — Section VI.
- *b.* In determining dry weight :
  - (1). In drying .... Section VII.
  - (2) In weighing.

ii. Errors introduced in the comparison of two halves of a leaf :
- *a.* Through asymmetry due to :
  - (1) Unsymmetrical venation ; }
  - (2) Inequalities in thickness ; } .... Section IV.
  - (3) Differences in composition.................... Section III.
- *b.* Through changes taking place in the course of experiment :
  - (1) In area .... Section V.
  - (2) In composition, including change in power of retaining water when dried .... Section III.

iii. Errors affecting the interpretation of the observed increase, due to :
- *a.* The heterogeneous composition of the increase—starch, sugars, proteids, oils, etc.................... Section III.
- *b.* The occurrence of respiration and translocation.

Section III.—On Changes of Composition during Insolation.

To ascertain whether any of the observed increase of dry weight during insolation is due to fixation of water, the *gain of carbon* per unit of area was determined in the same half-leaves in which the increase of dry weight had been carefully measured. For this purpose the "experimental" and "control" pieces of leaf were analysed by combustion, and the gain in carbon content per unit of area determined by the difference.

The analyses were carried out in the Caius College Chemical Laboratory, and my thanks are due to Dr. Ruhemann, of Caius College, for placing his apparatus at my disposal, and giving me every facility.

If photosynthesis resulted in the accumulation of some known single substance, the increase of dry weight could be accurately calculated from the gain of carbon. As, however, starch, various sugars, proteids, and even oils may be produced, any basis for calculation must be in some degree arbitrary, and perfect correspondence between the calculated increase and that actually observed is not to be expected.

There are, besides, other minor sources of discrepancy. The apparent gain of dry weight which would result from any shrinkage in area of the experimental half-leaf would have the composition of the general leaf

substance, and this might be quite different from the composition of the true assimilatory increase. Experimental errors also are not negligible.

If, with all these disturbing factors, a close agreement holds between the observed increase of dry weight and the values calculated from the results of the carbon analyses, it is clear that fixation of water cannot play an appreciable part, still less produce an overwhelming positive error over and above the real assimilatory gain.

Errors from incomplete drying may be for the present regarded as negligible. They are considered in Section VII (p. 45), where the procedure finally adopted for drying the very hygroscopic material is described.

The following tables give the results of the combustion analyses and the dry-weight results with which they are to be compared :—

In Tables I to III the calculated value for the gain of dry weight has been obtained by finding the weight of starch which is equivalent to the observed gain of carbon, and adding to this the observed increase in the ash content.

As the ash consists largely of carbonate, a few specimens were analysed, using lead chromate to decompose the carbonates of the alkali metals not decomposed by heat. Tables IV and V give the results obtained. The calculated value for the increase of dry weight is there simply the starch equivalent of the observed gain of carbon.

The essentials of the conditions under which the material analysed was obtained are given above each table.

The examples of *Helianthus tuberosus* in Table I were carefully powdered before the final drying and combustion. The results in the other tables were obtained with material which had been dried in a form convenient for immediate analysis.*

The entire half-leaves were employed for the experiments in Table I and their area found by applying a planimeter to photographic prints ;† the results are therefore all calculated for an area of one square decimetre.

Where equal areas from each half-leaf were cut, by means of templets,† such as were used by Sachs, or by the rotating punch† described on p. 42, no such calculation was necessary, and for convenience in estimating the degree of accuracy the results have been left in terms of the actual area used. This area is given for each experiment.

Where the punch method was used the number of pieces punched from each half-leaf is given ; each piece measured 0·804 square centimetre. In one or two cases the numbers of discs from the two halves were not identical, and in these examples the figures for one half are calculated for the number of discs used in the other half.

* See p. 45.

† The various methods of area determination are described in Section VII. For brevity they will be referred to as the planimeter, templet, and punch methods respectively.

Table I.—*Helianthus tuberosus*: area of entire half-leaves by planimeter method.

Assimilation experiment; August 23, 1906. Leaves attached to plant; darkened previous evening. Intermittent sun. Time, $7\frac{1}{2}$ hours: 10 A.M. to 5.30 P.M. a = control half-leaf; b = experimental half-leaf.

Numbers are grammes per square decimetre.

Leaf.	Area in sq. cm.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.	Ash found on combustion.	Gain of ash.	Calculated gain of dry weight.
1	(*a*) 26·9 (*b*) 26·8	0·378 0·417	**0·039**	0·633 0·694	0·061	0·037	0·049 0·053	0·004	**0·041**
2	(*a*) 60·2 (*b*) 58·5	0·342 0·365	**0·023**	0·556 0·603	0·047	0·029	0·040 0·043	0·003	**0·032**
3	(*a*) 77·5 (*b*) 85·5	0·399 0·393	**−0·006**	0·618 0·611	−0·007	−0·005	0·064 0·063	−0·001	**−0·006**
4	(*a*) 80·2 (*b*) 77·8	0·446 0·470	**0·024**	0·671 0·704	0·033	0·019	0·084 0·086	0·002	**0·021**

Table II.—*Helianthus tuberosus*: area by templet method.

Assimilation experiment; August 28, 1907. Leaves detached, in greenhouse; temperature 25°—27° C. Sun, under canvas. Time 8.30 A.M. to 3.30 P.M.: 7 hours.

Numbers are grammes, and refer to the given areas.

Leaf.	Area in sq. cm.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.	Ash found on combustion.	Gain of ash.	Calculated gain of dry weight.
1	50	(*a*) 0·1465 (*b*) 0·1668	**0·0203**	0·2324 0·2665	0·0361	0·0209	0·0236 0·0267	0·0031	**0·0240**
2	50	(*a*) 0·1513 (*b*) 0·1622	**0·0109**	0·2518 0·2651	0·0133	0·0082	0·0195 0·0219	0·0024	**0·0106**
3	50	(*a*) 0·1449 (*b*) 0·1597	**0·0148**	0·2333 0·2556	0·0223	0·0137	0·0236 0·0267	0·0031	**0·0168**
4	40	(*a*) 0·1223 (*b*) 0·1297	**0·0074**	0·1952 0·2080	0·0128	0·0088	0·0222 0·0223	0·0001	**0·0089**
5	40	(*a*) 0·1284 (*b*) 0·1346	**0·0062**	0·2046 0·2150	0·0104	0·0064	0·0209 0·0232	0·0023	**0·0087**

Table III.—Cherry Laurel: area by disc method.

Assimilation experiment; August 29, 1907. Leaves detached, in glass case supplied with large excess of CO_2. Five hours of bright sunshine. A, fully insolated; B, shaded by double thickness of wet cotton cloth.

Numbers are grammes, and refer to the particular number of discs of leaf lamina.

Leaf.	No. of discs.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.	Ash found on combustion.	Gain of ash.	Calculated gain of dry weight.
A 2	14	(*a*) 0·1245 (*b*) 0·1316	**0·0071**	0·2192 0·2362	0·0170	0·0104	0·0085 0·0073	−0·0012	**0·0092**
3	15	(*a*) 0·1097 (*b*) 0·1119	**0·0022**	0·1919 0·2000	0·0081	0·0049	0·0062 0·0055	−0·0007	**0·0042**
4	15	(*a*) 0·1064 (*b*) 0·1162	**0·0098**	0·1861 0·2035	0·0174	0·0107	0·0066 0·0068	+0·0002	**0·0109**
B 2	15	(*a*) 0·1314 (*b*) 0·1286	**−0·0028**	0·2346 0·2246	−0·0100	0·0061	0·0067 0·0068	+0·0001	**−0·0060**
3	12	(*a*) 0·0861 (*b*) 0·0925	**0·0064**	0·1520 0·1655	0·0135	0·0083	0·0052 0·0050	−0·0002	**0·0081**
4	15	(*a*) 0·1267 (*b*) 0·1318	**0·0051**	0·2227 0·2304	0·0077	0·0047	0·0068 0·0075	+0·0007	**0·0054**

Analyses with Lead Chromate (to drive off CO_2 from ash).

Table IV.—Cherry Laurel: area by disc method.

Assimilation experiment; August 16, 1907. Leaves detached, in glass case supplied with large excess of CO_2. Time of exposure 11 A.M. to 6 P.M., 7 hours. Occasional sunny intervals, temperature in case varying from 18° to 27° C. according to illumination.

Numbers are grammes, and refer to the given number of discs.

Leaf.	Number of discs.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.
1	12	(*a*) 0·1070 (*b*) 0·1177	**0·0107**	0·1970 0·2126	0·0156	**0·0096**
2	12	(*a*) 0·0931 (*b*) 0·1042	**0·0111**	0·1682 0·1828	0·0146	**0·0090**
3	10	(*a*) 0·0805 (*b*) 0·0950	**0·0145**	0·1465 0·1701	0·0236	**0·0145**
4	9	(*a*) 0·0731 (*b*) 0·0784	**0·0053**	0·1302 0·1392	0·0090	**0·0055**
5	10	(*a*) 0·0866 (*b*) 0·0941	**0·0075**	0·2689 0·1717	0·0156	**0·0096**

Table V.—*Helianthus tuberosus*: area by disc method.

Assimilation experiment; August 19, 1907. Leaves detached, in greenhouse, under canvas. Time of exposure, 11.45 A.M. to 5.45 P.M. Intermittent sun. Temperature 23° C.

Number of discs.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.
65	(*a*) 0·1701 (*b*) 0·1923	**0·0222**	0·2689 0·3020	0·0331	**0·0203**

In Table VI two examples are given, in which asymmetry in dry weight and carbon content have been compared, to indicate the degree of accuracy obtained and the kind of experimental error to be allowed for in considering the results already given.

Table VI.—Cherry Laurel: Examples of Asymmetry.

All numbers are grammes, and refer to the actual area used.

a = left half-leaf. *b* = right half-leaf.

	Dry weight.	Ash.	Dry weight, less ash.	Percentage difference in organic substance.	CO_2 found on combustion.	Percentage difference in CO_2.
1	(*a*) 0·1044 (*b*) 0·1024	0·0051 0·0054	0·0993 0·0970	2·3	0·1927 0·1868	3·2
2	(*a*) 0·1541 (*b*) 0·1565	0·0080 0·0074	0·1461 0·1491	2·1	0·2783 0·2853	2·5

The differences in these percentages are of similar magnitude to the discrepancies in the previous tables, and it appears, therefore, that on the whole there is little in the results given in those tables to be explained otherwise than by errors incident to the technique of experiment. What differences there are, moreover, do not afford any explanation whatever of the large positive error found by Brown and Escombe in results obtained by the dry-weight method. The differences are small, and are for the most part in favour of the apparent gain of carbon. Indeed, in Tables I to III as a whole, the observed increase in dry weight is less than that calculated from the gain of carbon and ash. This might be due to the accumulation of substances containing a higher percentage of carbon than starch; or to

some extent, especially with *Helianthus*, to shrinkage of the experimental half-leaf; for the leaf substance, as a whole, contains about 50 per cent. of carbon, whereas starch contains only 44 per cent.

Only in one instance is the difference too great to be easily explained (Table I, Leaf 2). Here it is possibly due to an exceptional experimental error.

Composition of the Gain of Dry Substance.

Some further points of interest connected with these analyses require consideration. In the first place, they suggest inorganic substance as a possible constituent of the gain of dry weight. It is not certain how far the observed changes in ash content were real, or how far only apparent and due to shrinkage; but differences of 12 or 13 per cent. (*cf.* Table II) are far too large to be reasonably accounted for by shrinkage. Moreover, an accumulation of inorganic substance is to be expected, at any rate in leaves attached to the plant and in detached leaves supplied with nutritive solutions.* It is also probable that these substances are translocated.

Broocks† and Menze‡ have also published ash analyses which tend to support these suggestions, although, again, the interpretation of their results is rendered somewhat doubtful by the possible occurrence of shrinkage. Hence, until more conclusive experiments have been carried out, it is necessary to bear in mind that changes of dry weight may consist in part of ash, perhaps to the extent of 5 per cent. or more, and in using Sachs' method it is advisable, for accurate work, to determine the ash content of the dried leaf material and deduct it from the dry weight.§

Having thus obtained the increase in organic substance, there still remains

* The analyses in Table II suggest that even when detached leaves are supplied with distilled water an accumulation of mineral substance in the lamina may take place, presumably by translocation from the stalk and principal veins.

Menze's ash analyses may also indicate a similar translocation: he used detached leaves of trees like *Platanus*, *Tilia*, *Quercus*, exposed them to diffused light only, and protected them from wind by inverted beakers, so that it is more probable that there was some expansion than that much shrinkage took place. In the case of those leaves which were exposed in closed vessels of air free from carbon dioxide, the apparent fall in ash content observed was probably due to a positive area change.

† Broocks, W., "Über tägliche und stündliche Assimilation einiger Kulturpflanzen," 'Inaug.-Diss.,' Halle, 1892, pp. 17—20.

‡ Menze, O., "Zur Kenntniss der täglichen Assimilation der Kohlehydrate," 'Inaug.-Diss.,' Halle, 1887, pp. 10—12.

§ The ash content in Table III for Cherry Laurel shows irregular fluctuations; but the quantities dealt with were small, and the changes themselves scarcely exceed, in most cases, possible experimental errors. Cherry Laurel transpires but slowly, and a large increase would therefore not be expected.

uncertainty as to its composition, and hence as to the amount of carbon dioxide which it represents.

The fact illustrated by the analyses, that the increase of dry weight corresponding to a given gain of carbon may be less than if it were all composed of starch, justifies the use, in this investigation, of the starch equivalent of the gain of carbon in preference to the carbohydrate equivalent as calculated by Brown and Escombe's "carbohydrate factor,"* which would have given a still higher value.

Brown and Escombe arrived at this factor, for reducing carbon dioxide absorbed to its equivalent gain of dry weight, from data in Brown and Morris' determinations of the relative amounts of starch and various sugars present in leaves of *Tropæolum majus* after vigorous assimilation. Besides leaving out of account that Brown and Morris did not determine the *increase* in sugars and starch in these experiments,† it does not allow for the probable accumulation of substances other than carbohydrates.

In particular there is evidence that proteid formation occurs in assimilating leaves.‡ Saposchnikoff's researches‡ are of especial interest in the present connection. He experimented with leaves assimilating normally, and, using the half-leaf method, determined the dry weight, carbohydrates, and proteid of the same portions of leaf material. His figures indicate that the increase in proteid may account for a considerable percentage of the increase of dry weight.

Since proteids contain a greater percentage of carbon than even starch, the total increase in dry weight must be less than if no proteid were formed at all. The "proteid factor" analogous to Brown and Escombe's carbohydrate factor ($= 0{\cdot}64$) is about $0{\cdot}54$. Taking their carbohydrate factor for the carbohydrates themselves and supposing that the increase of proteid were equal to one-third of the increase in carbohydrates, as was the case in some of Saposchnikoff's experiments,§ the true "dry-weight factor" would be 0·61, or practically equal to the "starch factor."

The true factor may vary considerably with conditions. One of Saposchnikoff's experiments§ indicates that, given (1) a comparatively dull

* *Loc. cit.*, p. 43.

† Brown and Morris, "Chemistry and Physiology of Foliage Leaves," 'Journ. Chem. Soc.,' vol. 63, 'Trans.,' 1893, p. 604.

‡ *Cf.* Menze, *loc. cit.*; Crapowicki, "Eiweissbildung in den chlorophyllführenden Pflanzen," 'Bot. Cent.,' 1889, vol. 39, p. 536; Saposchnikoff, "Eiweissstoffe und Kohlehydrate der grünen Blätter als Assimilationsproducte," 'Bot. Cent.,' 1895, vol. 63, p. 246; Zaleski, "Eiweissbildung," 'Ber. d. D. Bot. Ges.,' vol. 15, 1897, p. 536; Palladin, "Influence de la Lumière sur la Formation des Matières protéiques actives et sur l'Énergie de la Respiration des Parties vertes des végétaux," 'Rev. gén. de bot.,' vol. 11, 1899, p. 81.

§ *Loc. cit.*, p. 247.

light so that the rate of formation of carbohydrates may not be great, and (2) an abundant supply of nitrates, practically the whole of the increase may take the form of proteid. In another experiment, in which distilled water was supplied to leaves instead of a nutritive solution, there was, if anything, a slight diminution in the amount of proteid present.*

The following table illustrates the possible range of variation in the weight of substance which might result from a given intake of carbon dioxide:—

Table giving Weights of various Substances which contain 12 grammes of Carbon, *i.e.* which are equivalent to 44 grammes of CO_2.

	Grammes.
Carbohydrates—	
Starch, $C_6H_{10}O_5$	27
Cane sugar, Maltose, Inulin — $C_{12}H_{22}O_{11}$	28·5
Hexoses, $C_6H_{12}O_6$	30
Ethereal oils, etc.—	
$C_{10}H_{16}$, $C_{10}H_{16}O$, etc.	14—15
Nitrogenous substances—	
Proteins	22—24
Amino acids—	
Asparagin	33
Leucin	21·8
Tyrosin	20·1

It appears from this table that the error involved in the assumption that the whole increase is of the average composition of starch might possibly be large. Fortunately, published experiments show that the substances which usually form the greater part of the increase are limited to those containing 40 to 50 per cent. of carbon, and that several of these are usually present, of differing composition, so that the average composition shown by the increase probably varies within narrower limits. Taking this smaller series of products, the weight corresponding to 12 grammes of carbon varies between 24 and 30 grammes. This means an extreme error, if the "*starch* factor" is used, of $\pm$ 3 grammes in 27 grammes, or about 10 per cent.

Variations of this kind may occur in the same plant under different conditions, and certainly occur in different plants, so that an error is involved in comparative investigations of all kinds, and the results will therefore only be approximate. But, as the analyses show, there is sufficient

* *Loc. cit.*, p. 247. Since, in assimilation experiments, detached leaves are usually supplied with distilled water only, a decrease in proteid might be expected from this experiment of Saposchnikoff. The composition of the increase would thus be different for attached and detached leaves.

correspondence between the increase in dry weight and the starch equivalent of the gain of carbon to enable a good deal of wide comparative work with varying plants and conditions to be done by the dry-weight method without much vitiation by such errors of interpretation.

For more exact work a convenient and rapid method of carbon analysis would be a distinct advantage. The wet-combustion method described by Hall, Miller, and Marmu,* depending on the absorption and estimation of carbon dioxide by the method elaborated by Brown and Escombe,† has been suggested to me by Dr. F. F. Blackman as possibly adaptable to the purpose. Besides rapidity and convenience, it would have the advantage that the whole of the carbon is driven off from the ash as well as from the organic material, and ash determinations would be avoided. However, the possibility of using this method has still to receive further consideration, especially in respect to the small quantity of material which can be analysed, owing to the limited amount of carbon dioxide that a Reiset tower will efficiently absorb. It must suffice here to point out that along some such lines modifications may be possible which would avoid all the errors of interpretation that have their origin in differences of composition of the photosynthetic products.

Section IV.—The Error from Lack of Symmetry.

This source of error, which Brown and Escombe conclude to be serious, Sachs‡ apparently assumed to be negligible when dealing with corresponding patches on either side of the midrib of a leaf.

He realised, however, the necessity of taking precautions to ensure as great a degree of similarity as possible between the pieces of material to be compared. The same templets were used on the two sides of the leaf, and care was taken to include as nearly as possible the same proportion of veins in corresponding pieces, and where possible to avoid outstanding veins altogether. The leaves were also carefully selected for their freedom from blemishes, such as dry spots or crumpled areas.

Sachs also used a number of leaves for each experiment, and so reduced the probable error from asymmetry, though he does not make a point of this.

Menze, experimenting with comparatively small leaves, had to use the greater part of each half-leaf, and could not select similarly veined portions.

* "The Estimation of Carbon in Soils and Kindred Substances," 'Journ. Chem. Soc., vol. 89, 'Trans.,' 1906, p. 595.

† 'Phil. Trans.,' B, vol. 193, 1900, p. 289.

‡ *Loc. cit.*

He was led in consequence to take particular notice of apparent symmetry, and concluded that it was impossible to find completely symmetrical leaves. Not only was dissimilar distribution of veins sometimes impossible to avoid, but differences in thickness were observed; and these could not be taken into account in selecting leaves for experiment.* Nevertheless he made no measurements of the extent to which such differences could affect his results.

When Brown and Morris† made the first actual determination of the effect of such asymmetry, the difference was found to be considerable, and of a higher order of magnitude than the errors of weighing and of measuring area, the only errors mentioned and estimated by Sachs. They aimed at repeating Sachs' procedure as closely as possible, and, using seven leaves of *Helianthus annuus*, found a difference of 0·43 gramme per square metre (*i.e.* 1·1 per cent.) between the portions from right and left halves respectively. Great care was taken in this experiment to choose pieces as similarly veined as possible, which was made more easy by the use of glass templets.

If fewer leaves and smaller areas were employed the error could be considerably greater than that found by Brown and Morris using seven leaves and 800 square centimetres. Brown and Escombe's results for single leaves illustrate this. One of their *Catalpa* leaves showed a difference of nearly 6 per cent. Their results may be quoted here for comparison with others to be given later.

Brown and Escombe's Asymmetry Determinations.‡

	Percentage difference.
Catalpa bignonioides	−3·9
	−4·3
	+2·3
	−5·7
	−0·7
Average	±3·4
Catalpa purpurea	+2·5 [misprinted +2·0]
Catalpa Bungei	+1·4
	−2·3 [" −1·7]
Tropæolum majus	+0·4
Polygonum Weyrichii	−1·1 [" −0·1]
Mean of all the results	±2·4 [" ±2·2]

* *Loc. cit.*, p. 12.

† *Loc. cit.*, p. 625.

‡ *Loc. cit.*, p. 60, Table IX.

In these determinations the area of the whole of each half-leaf was measured by the planimeter method. A number of prominent veins were therefore included, and careful examination of a number of *Catalpa* leaves, even if chosen for their apparent symmetry, reveals the fact that the venation is not symmetrical. It seemed unlikely that the mesophyll itself would exhibit differences as great as those found by Brown and Escombe for mesophyll *plus* veins. For this reason modifications in the methods of area determination have been adopted* with the object of avoiding all outstanding veins. The rotating punch (p. 42) is very useful in this respect, owing to the small size of the discs it will accurately cut, although it is not so rapid in use as templets. In certain cases small templets have also been used.

The advantage of avoiding veins altogether is clearly shown by some of the results given in the tables that follow. For instance, the average difference for 11 leaves of *Helianthus* when the punch method was used (Table VIII) was $\pm$ 1·4 per cent., whereas four leaves for which the templet method was used, although the main veins were avoided, showed the considerably higher average difference of $\pm$ 2 per cent.†

The experiment with *Paulownia imperialis* (Table XIII) gave results still more striking. The large leaves of this tree are strongly veined, but between the main veins are areas practically without projecting veins. From these areas pieces can be cut with templets as small as 4 cm. by 2·5 cm., with considerable accuracy. Towards the margin, especially near the base, are other areas traversed by the slightly projecting ultimate branches of main veins, but these are nevertheless not prominent enough to increase appreciably the errors of cutting. The contrast between the differences shown by pieces including veins, and by other pieces from the "veinless" areas, is instructive. For the latter the average difference was $\pm$ 1·4 per cent., for the former $\pm$ 5·9 per cent., more than four times as much.

The results for *Catalpa bignonioides* (Table XIV), obtained by the templet method without avoiding veins, correspond fairly well with Brown and Escombe's results for the same plant.‡ On the whole, omitting leaf 5, where the extreme difference was due to the slight convexity of one half, the differences are rather less than theirs.

The other tables give the results of experiments with other leaves.

The general plan of dealing with individual leaves has been followed in order to determine how far the different results given by individual leaves in the same assimilation experiment were to be accounted for by asymmetry, and how far other sources of

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* See Section VI, pp. 39—44.

† Errors involved in area determination by templets may account for part of this difference. *Cf.* Section VI, pp. 39, etc.

‡ Quoted on p. 13.

error were to be reckoned with. The leaves used were of several different types: Cherry Laurel, thick and somewhat leathery; *Helianthus tuberosus*, thin but coarse, and *Tropæolum majus*, thin and delicate, both without a strongly supporting venation; Lime and *Cercis*, similarly contrasted as regards delicacy but with a very efficient supporting network of veins. The templet, planimeter, and punch methods were all used in determining areas.

Attention has been concentrated on *Helianthus tuberosus* and the Cherry Laurel, since they were also used for assimilation experiments.

TABLES SHOWING DEGREE OF ASYMMETRY EXISTING IN VARIOUS LEAVES.

Explanatory Notes.

The differences given were obtained by subtracting the dry weight of the right half-leaf from that of the left, and the percentages have been calculated in terms of the latter. The percentage differences are given in column 2, the actual differences in milligrammes per square decimetre in column 1.

In each table, either the range of areas used in the series of experiments is given at the head of the table, or the actual area cut from each half-leaf is given, in a special column, for each experiment. Where the rotating punch* was used, the number of discs is given instead of the area. The area of each disc was 0·804 square centimetre.

In some cases the number of discs cut from each side was not the same: this was due to the slightly unequal area of the two sides, or to different arrangement of the veins, which were avoided where possible. For instance, in using the disc method with leaves of *Helianthus tuberosus*, care was taken to make the discs equally representative of the mesophyll on either side, whereas "symmetrical" cutting relative to the midrib would have been impossible. This applies also to some of the experiments with Cherry Laurel. The lateral veins in the larger leaves were sufficiently distant from one another to allow of discs being cut from between them: absence of perfectly symmetrical placing of the veins led to the cutting of different numbers of discs in such cases. Later, the supply of large leaves was exhausted, and the smaller leaves had less prominent veins, so that there was less objection to including them. Discs were then cut in equal numbers and from symmetrical positions from the two sides.

Table VII.—Cherry Laurel. Cut by rotating punch.

Number of discs from each half-leaf varied with size of leaf from 10 to 25.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	−12	−1·3
" 2	−25	−2·8
" 3	−16	−2·0
" 4	+27	+2·5
" 5	+ 1	Nil
" 6	−25	−3·0
" 7	− 8	−1·1
" 8	+26	+3·3
Averages of 1—8	±17	±2·0
Leaves 1—8 taken together	− 4	−0·5
Several leaves taken together—		
8 leaves—Experiment 9	− 1·9	−0·2
6 " " 10	−33	−2·7
7 " " 11	+17	+1·5
6 " " 12	+17	+1·4

* See p. 42.

Table VIII.—*Helianthus tuberosus.*

A.—Using the rotating punch: discs cut from between outstanding veins, 20 to 42 from each half-leaf. In the experiments with single leaves, the percentage differences are given to the nearest 0·5.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	Nil	Nil
" 2	−7	−3·5
" 3	Nil	Nil
" 4	−1	−0·5
" 5	+1	+0·5
" 6	−6	−2·5
" 7	+9	+3·0
" 8	−4	−1·5
" 9	+5	+2·0
" 10	−1	−0·5
" 11	+2	+1·0
Averages of 1—11	±3	±1·4
Leaves 1—11 taken together	Nil	Nil
4 small leaves taken together—Experiment 12	+1·9	+0.7

Table IX.—*Helianthus tuberosus.*

B.—By templet method: total area cut from each half-leaf given for each experiment. It was not recorded which dry weights correspond to right and left halves respectively.

		(1)	(2)
	sq. cm.	milligrammes.	per cent.
Single leaves—Experiment 1	20	8 5	2·6
" 2	70	8·7	2·6
" 3	20	5·5	1·6
" 4	40	4·0	1·4
Averages of 1—4	...	±6·7	±2·0

Table X.—*Tropæolum majus.*

A. Experiments 1—4. Area of entire half-leaves by planimeter method: areas between 47 and 64 sq. cm.

B. Experiment 5. By rotating punch: 50 discs from one half (whether left or right not recorded), 54 from the other; differing number due to different distribution of veins on the two half-leaves.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	+ 9	+3·8
" 2	− 6	−2·1
" 3	+12	+4·0
" 4	+ 4	+1·5
" 5	12	4·8
Averages of 1—5	± 9	±3·2

Table XI.—Lime.

A. Experiments 1 and 2. By planimeter method : area about 30 sq. cm.
B. Experiments 3 and 4. By rotating punch. Experiment 3, 10 discs ; Experiment 4, 13 discs.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	−13	−3·3
„ 2	+28	+4·4
„ 3	+15	+4·0
„ 4	+ 4	+1·0

Table XII.—*Cercis.* By rotating punch.

Number of discs 17—20.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	+15	+3·5
„ 2	+ 9	+2·5

Further Experiments on Asymmetry.

The experiments in Tables XIII and XIV were performed in connection with assimilation experiments. The leaves were cut from trees in the University Botanic Garden in the early morning, while they were still wet with dew, were carried to the laboratory, wrapped in a damp cloth in a vasculum, and used at once.

Templets were used in all the experiments.

Table XIII.—*Paulownia imperialis.*

Three leaves furnished material for six experiments ; in four of these, only pieces without prominent veins were cut, in the other two the pieces included minor outstanding veins.

The area is given for each experiment. Where two pieces were cut from each half-leaf for a single experiment, the areas of the individual pieces are connected by a plus sign.

Leaf.	Portion.		(1)	(2)
Avoiding veins.				
		sq. cm.	milligrammes.	per cent.
1	1	30	+ 9·7	+1·5
1	2	10+10	+ 9·5	+1·6
2	1	21+10	− 6·1	−0·9
3	1	21+10	+ 9·7	+1·6
Including veins.				
2	2	21+15	−39·7	−5·9
3	2	20	−33·5	−6·0

	Avoiding veins.	Including veins.
	milligrammes.	milligrammes.
(1) Average difference per square decimetre ...	±9	−37
Difference per square decimetre taking all together	+5	−37
(2) Average percentage difference	±1·4	± 5·9
Percentage difference taking all together ...	+0·8	− 5·9

Table XIV.—*Catalpa bignonioides.*

Except in the case of Leaf 1, the midrib was removed before cutting pieces from the half-leaves with the templet: area in each case 50 sq. cm.

Leaf.	(1)	(2)
	milligrammes.	per cent.
1	+19·0	+4·0
2	− 1·6	−0·3
3	+10·6	+1·7
4	−10·6	−1·8
5	+38·8	+8·1
6	−17·8	−3·1
The left half of Leaf 5 was seen to be not perfectly flat; the results for this leaf have therefore been omitted from the following averages.		
	Excluding Leaf 5.	
Average	±11·9	±2·2
Taking all together	− 0·1	Nil

The results as a whole prove the necessity for determining this error for each plant used in an investigation. Asymmetry, to the extent of an average of $1\frac{1}{2}$ to 3 per cent., is shown by all the leaves examined, and in extreme cases it varies from zero to 4 per cent. or more. *Catalpa bignonioides* shows a higher degree of asymmetry than the average.

The seriousness of this asymmetry in assimilation experiments is greater in thick than in thin leaves. Thus in Cherry Laurel, in an experiment lasting seven hours, asymmetry to the extent of 2 per cent. will mean an error of about ± 3 milligrammes per square decimetre (± 0·3 gramme per square metre) per hour, while in *Helianthus tuberosus* it will be less than ± 1 milligramme per square decimetre per hour. For a rate of increase of 10 milligrammes per square decimetre per hour, such as Brown and Morris found for detached leaves of *Helianthus annuus*, the average percentage error would be 30 per cent. in Cherry Laurel, 10 per cent. in *Helianthus*.

By using a number of leaves this error may be reduced roughly in the

inverse ratio of the square root of the number of leaves used. In Brown and Morris's example, for instance, the asymmetry of seven leaves of *Helianthus annuus* taken together was 1·1 per cent. Some similar results for *Helianthus tuberosus* and Cherry Laurel will be found in the tables, though with leaves of the latter the error may still be high (*e.g.* 2·7 per cent. for six leaves in Experiment 10). Further discussion of these results will be postponed till the errors as a whole are discussed.*

Attention may be called here to the fact that all asymmetry determinations are affected by errors classed above as errors of technique; but, as will be shown later,† these are as a rule relatively small, and account for only a small part of the differences shown in the tables.

Section V.—On Change of Area during Experiment.

Brown and Escombe's conclusion, that the results given by the dry-weight method are too high, was based not only upon their direct test with Catalpa, but also on a consideration of the general high level of the results obtained by themselves and others when using it. In particular, the value found by Sachs for the rate of assimilation in detached leaves of *Helianthus annuus* was far higher than any which they observed in their experimental chamber.‡

If the method itself is really responsible for these high values, some change, related in a definite way to the conditions of experiment, must take place in the experimental half-leaf.

Of such possible changes, that of water fixation suggested by Brown and Escombe has been disposed of in Section III, where it is shown that Sachs' results would have been approximately the same had he measured *carbon content* instead of dry weight, all other details of his method remaining the same.

On the other hand, shrinkage in area due to loss of water could produce errors consistently in a positive direction, for the conditions under which a leaf might be expected to assimilate most rapidly are just those conditions which are favourable to increased evaporation.

For instance, Broocks§ observed a much greater increase of dry weight in fully insolated leaves of the Sugar Beet than in leaves shaded from direct sunlight. If the more rapid evaporation to be expected in the sun resulted in appreciable shrinkage, the whole or part of this difference might be only

* Section VIII, p. 48.

† Sections VI and VII.

‡ Sachs' result was 16 milligrammes per square decimetre per hour (*loc. cit.*, p. 25). The highest rate found by Brown and Escombe for *H. annuus* was 5·5 milligrammes (*loc. cit.*, p. 44).

§ *Loc. cit.*, p. 17.

apparent: it is, in fact, uncertain whether either intensity of light or leaf temperature were limiting factors to assimilation in these experiments of Broocks.

Again, the conditions under which dry-weight assimilation experiments have usually been conducted would tend to exaggerate errors from shrinkage. In order to ensure that the leaves are starch-free to begin with, they are covered with tinfoil overnight, or else the experiment is commenced soon after sunrise. In either case the leaf is likely to be in a condition of maximum turgidity at the beginning of the experiment. On the one hand, close confinement prevents the escape of moisture, often to such an extent that water vapour condenses on the inner side of the tinfoil; while on the other hand the relatively low temperature and high degree of humidity prevailing at night and in the early morning are also just the conditions to retard evaporation. Thus the area of the first half-leaf is measured, or pieces cut from it, immediately after treatment calculated to produce full turgidity. The other half is exposed to direct sunlight, or, even if the conditions are not so extreme as this, to conditions favourable to considerable evaporation. Hence the turgor might reasonably be expected to diminish and with it the area, and a positive error to be thus introduced.

Moreover, the frequent mention by previous workers of the difficulty of avoiding the wilting of detached leaves suggests that the much greater increase in dry weight shown in experiments with detached leaves might, in part at least, be only apparent. Even the results of translocation experiments would be too great, for the conditions under which these experiments are carried out are favourable to increase in turgidity, which would result in an apparent decrease in dry weight per unit of area and an over-estimate of the amount translocated. In addition to this, the leaves may still be slowly growing, and their growth expansion would introduce a further error in the same direction.

Sachs was aware of the danger of comparing turgid with flaccid leaves, and in his experiment with detached leaves of *Helianthus annuus* he floated the experimental halves on water for half an hour at the end of the experiment to make them turgescent. Yet neither Sachs, nor any subsequent experimenter, until Brown and Escombe published their results, gave any evidence of having tested whether shrinkage affected his own experiments appreciably; nor, it may be added, of having ascertained that the leaves used had ceased growing. They assumed that, so long as the condition of a leaf appeared to the eye to be approximately the same, such changes could not be great enough to affect their results to an appreciable extent.

The following experiment shows how unjustifiable this assumption was:—

A leaf of *Helianthus tuberosus* was allowed to dry up slowly, and at

several stages in the process records were made of its weight, apparent degree of turgidity, and linear dimensions.

The turgidity records are shown in fig. 2. They are rough diagrams indicating the positions assumed by the leaf when its stalk was placed (1) vertically, and (2) horizontally. Four stages are shown (A, B, C, D), beginning with Stage A, which represents the condition of the leaf after floating on water under a bell-jar till fully turgid.

The linear measurements were made with a millimetre scale between crosses marked

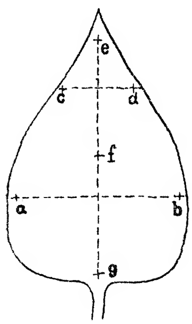

Fig. 1.

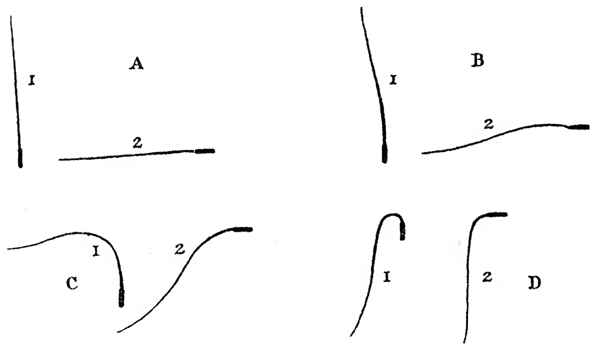

Fig. 2.

on the leaf with waterproof ink. Fig. 1 shows the disposition of the marks, and of the lengths measured.

In Table XV the records of weight and dimensions are given, for the stages indicated by the letters in the first column, in the order in which they were made. Stage A was twice returned to by floating the leaf on water.

The *changes* in the weight and dimensions from stage to stage are given in Table XVI, as percentages of their value at Stage A.

Table XV.

Lengths are given to nearest $\frac{1}{4}$ mm.

Turgidity diagram.	Dimensions in centimetres.				Fresh weight in grammes.
	a—b.	*c—d.*	*e—f.*	*f—g.*	
A	8 ·00	5 ·45	9 ·45	7 ·70	2 ·56
D	7 ·65	5 ·20	8 ·90	7 ·60	1 ·97
A	8 ·00	5 ·45	9 ·47	7 ·70	2 ·56
B	7 ·77	5 ·30	9 ·30	7 ·67	2 ·26
C	7 ·70	5 ·25	9 ·20	7 ·65	2 ·06
A	8 ·05	5 ·45	9 ·47	7 ·72	2 ·67

Table XVI.

Changes of dimensions from stage to stage as percentages of dimensions at Stage A.

	a—b.	*c—d.*	*e—f.*	*f—g.*	Decrease in area: estimated.	Loss of weight.
A—B	2·9	2·8	1·6	0·4	4	11·7
B—C	0·9	0·9	1·1	0·3	1·5	7·8
C—D	0·6	0·9	3·2	0·7	2·5	3·5
A—D	4·4	4·6	5·9	1·4	8	23·0

As far as external appearance went, Stage B was not readily distinguishable from Stage A, yet in passing from A to B the leaf had lost over 10 per cent. of its weight by evaporation, and had diminished in area by about 4 per cent. Passing on to C, a condition occurring quite commonly on the plant in the open air, there was a further loss of water of 8 per cent., and a much smaller change in area of about 1·5 per cent. From C to D the loss of water of only 3·5 per cent of the original fresh weight was accompanied by a rather greater loss of area of about 2·5 per cent., due to a greater shrinkage in length at this stage, and this brought the leaf to what may be described as a condition of complete flaccidity, such as occurs occasionally in hot sunny weather.

Summing up the changes, we find that between maximum turgidity and complete flaccidity the leaf decreased in weight by 23 per cent. through loss of water, and shrank by about 8 per cent. of its area.

Change of Area under Natural Conditions.—Helianthus annuus.

A large number of observations of a similar character have been made which show that the liability to shrinkage varies from leaf to leaf, with the character of the mesophyll itself, and with the nature of the epidermis and of the supporting venation. Most interesting of all is a series of observations on *Helianthus annuus* made during the period of fine weather at the end of July and beginning of August of 1908.

This plant is of especial interest, because it was with its leaves that Sachs obtained some of his highest values for the rate of assimilation. He commenced his experiment with *attached* leaves at 5 A.M., and continued it till 3 P.M., and my original object was to determine by direct measurement the change of dimensions which takes place between those hours on a bright sunny day.

Although the observations were begun soon after the ground had been

soaked with heavy rain, the amount of shrinkage in full sunlight was astonishing. Even more surprising was the sensitiveness of Sunflower leaves to changes in the intensity of the sunlight. In the following pages is given a selection of the results, which are of interest apart from the immediate purpose for which they were obtained.

Lengths were marked off on the leaves with fine black crosses over areas carefully selected for their flatness. They were measured with a millimetre scale : a support below the leaf, consisting of a flat rule covered with plush, yielded to the outstanding veins and allowed the upper surface of the leaf to remain flat under the scale.

Since the distances measured were of the order of 10 cm., a change of dimensions of 1 per cent. was easily detected.

Measurements were taken both transversely and along the midrib. With a good flat leaf four measurements were made (as in Leaves I and V, in the following tables and diagrams), one across each half at its widest part, another right across the leaf nearer the tip, and a fourth along the midrib.

The percentage change of area was estimated by averaging the percentage changes in the cross measurements, and adding the percentage longitudinal change.

Most of the following observations were made on three plants growing in the University Botanic Garden. Six leaves were chosen, and are referred to as I to VI respectively. I, III, and IV were on the same plant and II on another plant in the same clump, in a fairly exposed situation. V and VI were on a plant in the north border, sheltered from the wind.

The curves in fig. 3 represent the percentage changes which took place in the area of Leaves I, II, and V over a period of seven days. The measurements were begun on July 22, at 5 A.M., the dimensions obtained on this occasion were taken as standards, and the variations subsequently observed were calculated as percentages of those standard dimensions. The percentage changes of area, estimated as described above, are plotted as ordinates against the times as abscissæ.

Examining first the curves* for July 22, which was a bright sunny day throughout, the remarkable fall in the curve for Leaf I, between 5 A.M. and noon, is very striking, and indicates a shrinkage in area of 5 per cent. Recovery was rather less rapid, and by 3 P.M. the area was still 3·2 per cent. less than at 5 A.M. The appearance of the leaf gave evident indications of a decrease in turgidity by waving of the lamina, which was not so fully extended as when quite turgid at 5 A.M. In addition to this the stalk curved over, so that the leaf hung downwards. Unless signs of relative flaccidity were being specially looked for, the position of the leaf might perhaps have been attributed to heliotropic curvature, and the waving of the lamina to its pendant position. In the early morning clear heliotropic movements were noted.

* It is scarcely necessary to remark that the curves themselves are only of rough pictorial value, and do not accurately indicate the actual changes that take place in the periods intervening between the actual measurements.

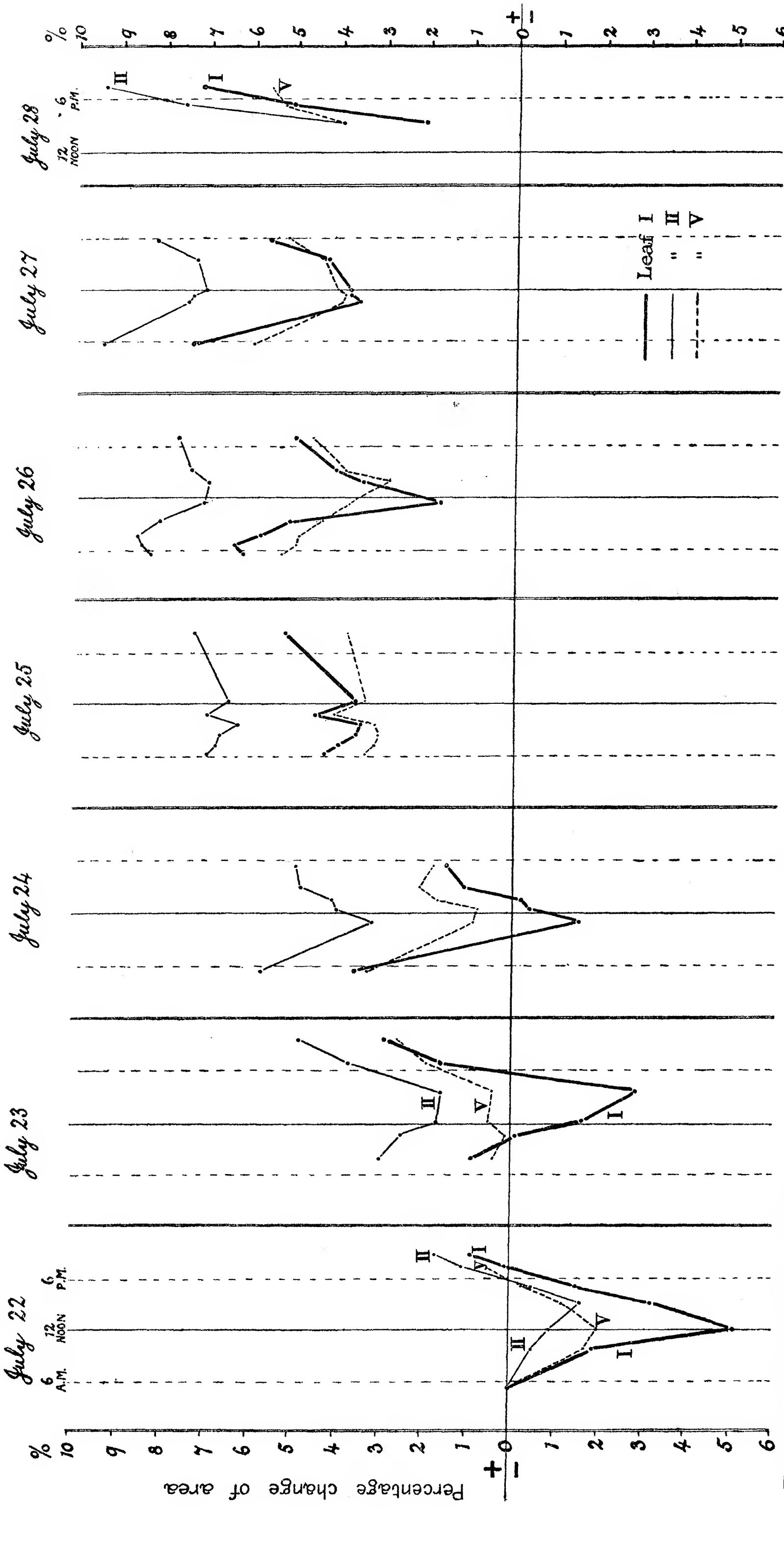

FIG. 3.—Curves showing changes of area of three leaves of *Helianthus annuus* during July 22 to 28, 1908. The ordinates are percentage differences from the area at 5 A.M. on July 22.

Leaves III and IV on the same plant (results not plotted) behaved in a similar way. Leaf II, on the other hand, appeared fully expanded and turgid right on till July 28, after a very trying windy morning. Even then so slight were the visible signs of change that, unless they had been very carefully watched for, the leaf would undoubtedly have been described as fairly turgid. Nevertheless, between 3 P.M. and 6 P.M., during its recovery, the increase in area was 5·4 per cent., and from 5 A.M. to 3 P.M. the shrinkage must have been at least 6 per cent.

The good condition of Leaf II was partly due to its sturdier construction, but later observations have shown that the ability to resist extreme conditions is largely a function of the age of the leaf. It was observed on August 7, under by no means extreme conditions, that Leaf I, which was situated fairly low on the plant, was completely flaccid, the lamina hanging in folds against the midrib. Higher up on the same plant, Leaves III and IV were only slightly waved, and towards the top of the plant were leaves which appeared completely turgid.

It was also observed that Leaf I became less and less able to withstand the heat of the sun, and although on July 22 it was only slightly waved at midday, yet on August 7, under conditions which were if anything less severe, it reached the completely limp state just described. The same phenomenon was even more strikingly shown by Leaf II, which up to July 27 had not shown the slightest signs of flaccidity to the eye. After the wind and sun of the 28th it quickly deteriorated and became early in August as unable as Leaf I to withstand bright sunshine without collapsing.

Referring for a moment to Leaf 5 it may be remarked how much less shrinkage this leaf shows than Leaf I. The curves for this leaf are much more comparable with those for Leaf II. This is to be attributed in part to similarity of age; in part, as the curves for July 28 show, to the more sheltered situation of Leaf V, in which it was protected on that day from the wind that continually agitated the plants in the open bed.

Fig. 4 shows details of all six leaves for a day of very varying illumination, haze and sun alternating as stated in the figure. Corresponding with these alternating periods of hazy and brighter weather, the slope of the curves for Leaves I and II changes from steep to nearly horizontal, indicating less rapid recovery in brilliant illumination. It will be observed that while Leaves III and IV show a similar alternation, Leaves V and VI behave differently, though they agree very closely indeed with each other. This difference is doubtless to be attributed to disturbing factors peculiar to the situation, but the records have not afforded any clue to the nature of those factors.

The rise in the curves for the following day, July 25 (fig. 5), shows in a very striking way the effect of clouds and rain. At 9.30 the sky was covered with thin grey cloud, through which the sun was only faintly visible, and from 10.15 to 10.35 a light shower of rain fell. During this

short period of little over an hour the leaves had all expanded to the extent of 0·7 to 1 per cent., and in the brighter interval which followed again decreased in area by nearly the same amount.

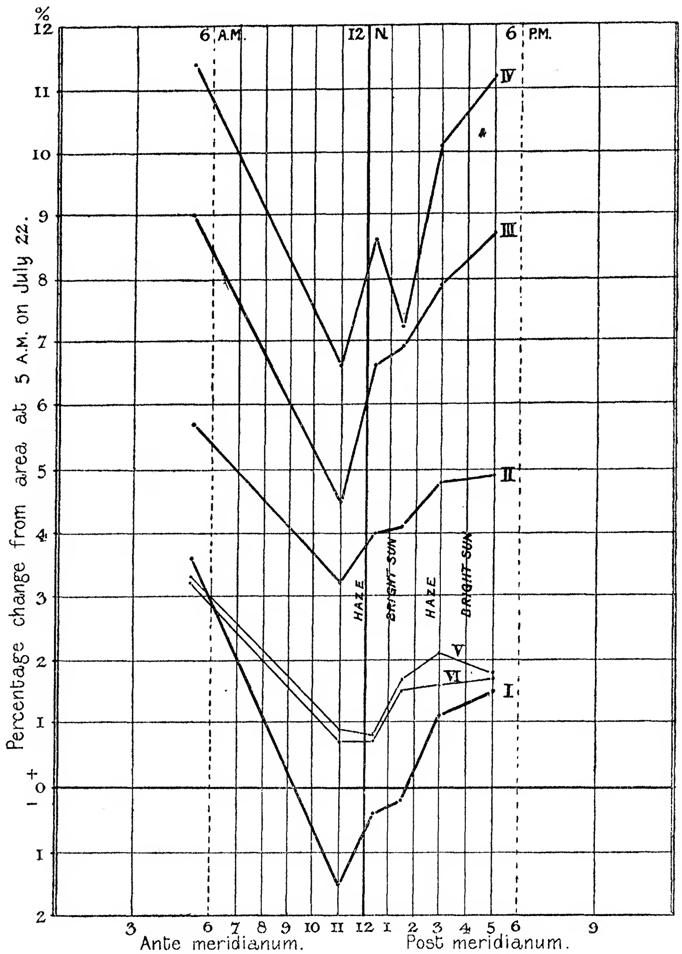

Fig. 4.—Curves showing more rapid expansion of leaves of *Helianthus annuus* when haze diminished the intensity of the sunlight. Bright sunshine prevailed until 11 A.M., two hazy periods occurred between 11 A.M. and 3 P.M., separated by an interval of bright sunshine lasting from 12.30 to 1.30 P.M., July 24, 1908. Ordinates are the percentage excess of the area over that at 5 A.M. on July 22.

These observations were followed up by a series of measurements made on a single leaf (Leaf III) between 10 A.M. and 12.20 P.M. on August 1, for the most part every 5 minutes. The curves (fig. 6) show the percentage changes in the linear dimensions, the continuous and the broken lighter lines corresponding respectively to transverse measurements of the left and right

halves of the leaf, and the heavy line to measurements along the midrib. The sky was somewhat cloudy, and periods in which clouds passed over the sun are indicated by shading on the right-hand side, corresponding to the

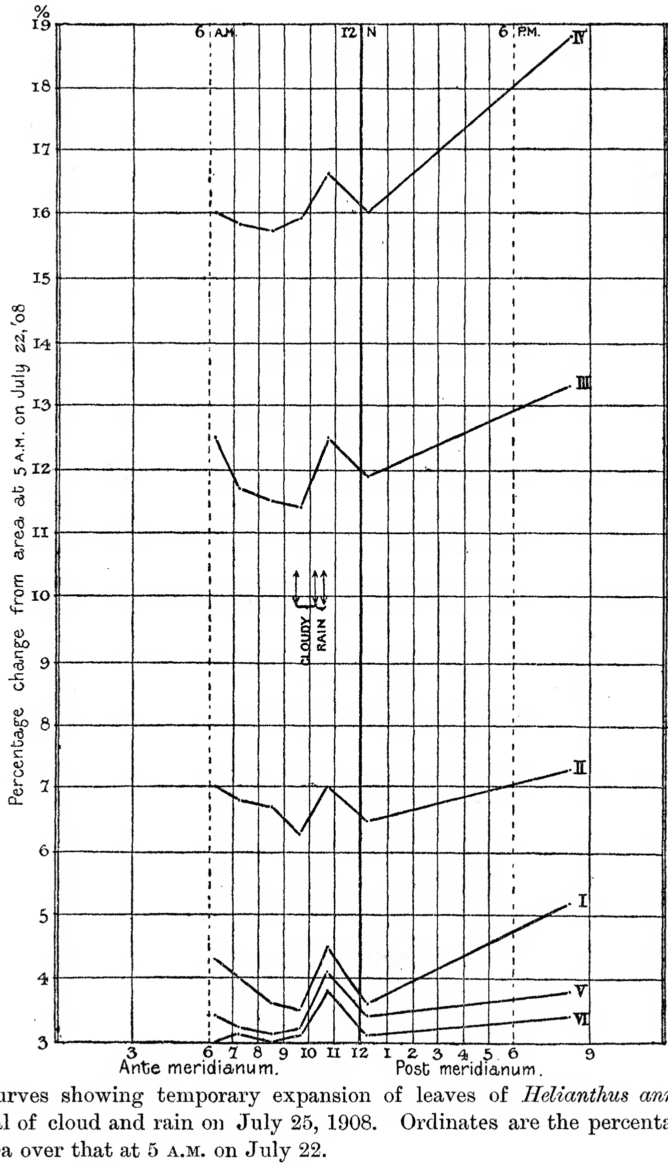

FIG. 5.—Curves showing temporary expansion of leaves of *Helianthus annuus* during interval of cloud and rain on July 25, 1908. Ordinates are the percentage excess of the area over that at 5 A.M. on July 22.

times which are shown on the left. The 5-minute observations commenced at 10.45, and the changes observed, as bright sunshine alternated with shade, were remarkably rapid. For instance, the left half of the leaf increased

1·6 per cent. in width in the 5 minutes elapsing between the measurements made at 10.50 and 10.55. It is highly probable that with more accurate and

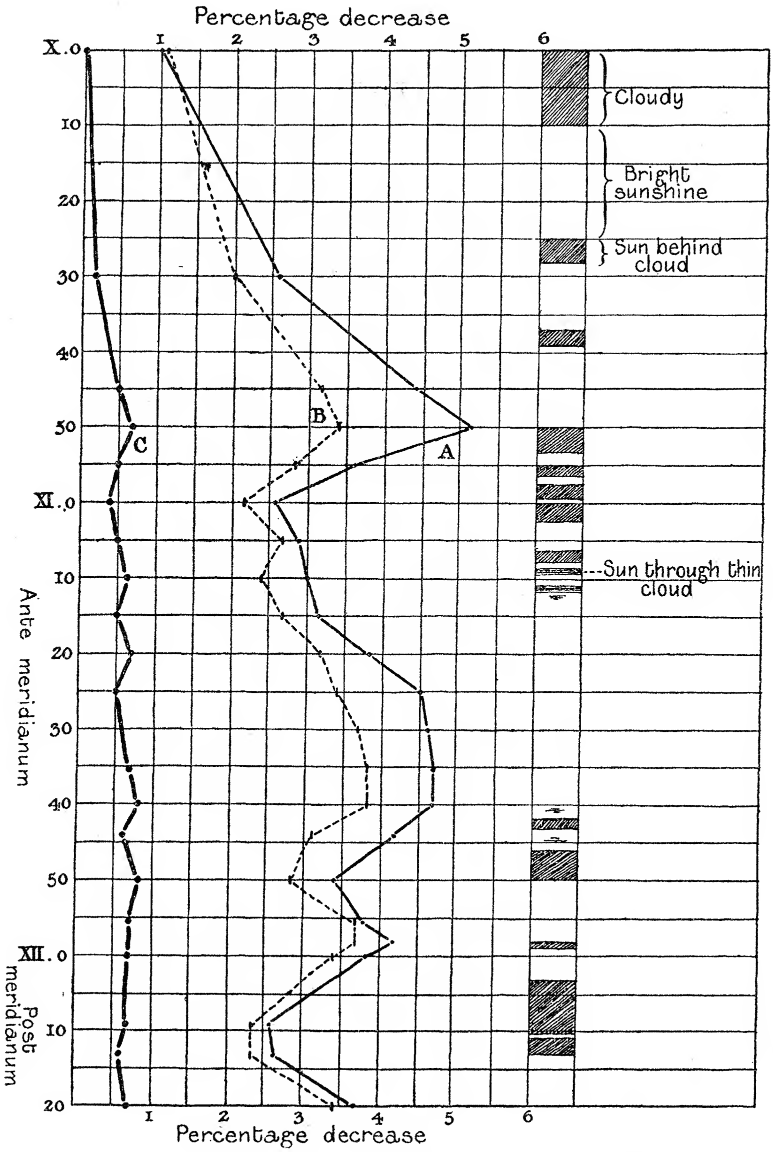

Fig. 6.—Curves showing the rapidity of reaction of a leaf of *Helianthus annuus* to varying illumination, on August 1, 1908.

Abscissæ are percentage differences from the linear dimensions in the early morning of the previous day. Curves A, B, C, refer respectively to measurements across the left and right half-leaves, and a measurement along the midrib.

continuous observations still greater sensitiveness and rapidity of reaction to changes of illumination would have been revealed.

It may be pointed out that the two halves of a leaf are not symmetrical even with respect to their change of dimensions with change of conditions. For example, between 10 and 10.50 A.M. the width of the left half of the leaf had shrunk 4·2 per cent., while the width of the right half at the same level had only decreased by 3·3 per cent. Similar differences were observed while making the measurements from which the curves on fig. 3 were constructed. It was also noticed that the leaf shrinks more and sooner, and recovers more tardily, towards the tip than it does nearer the base, a fact which is doubtless to be explained by relative distance from the water supply. As was to be expected from the nature of the tissues involved, the variations along the midrib are comparatively small, but, as shown also on fig. 6, correspond closely in sign with the variations in transverse dimensions. One other point is worthy of note. The right half of Leaf I showed a faster rate of growth from day to day than did the left half, as well as, if anything, a slightly smaller amount of shrinkage. With the possibility established of such differences as these between two sides of a leaf, a certain degree of asymmetry in the dry weight of the mesophyll itself is no longer surprising, although it does not necessarily follow that true growth expansion involves an alteration in dry weight per unit of area under comparable conditions of turgidity.

Fig. 7 shows the results of observations on Leaves 7, 8, and 9, belonging to plants growing on the south side of the University Botany School. The curve for Leaf I on the

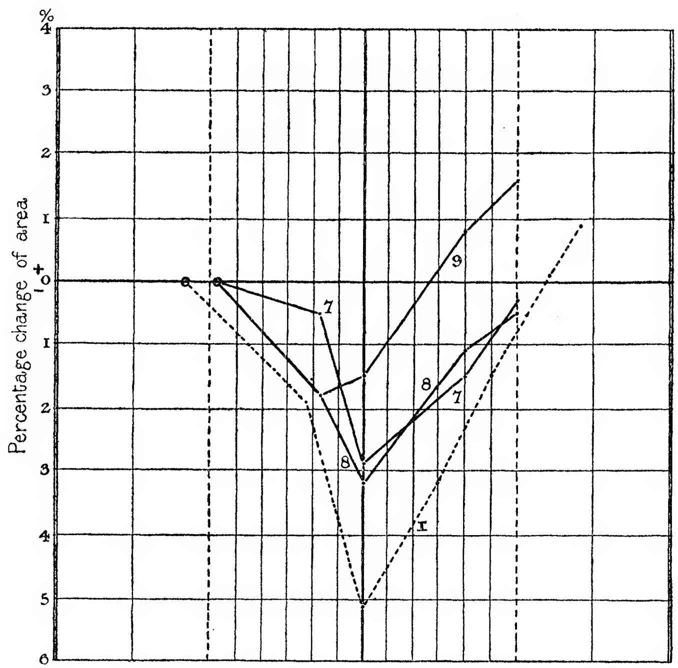

Fig. 7.—Shrinkage in area and recovery of leaves of *Helianthus annuus* on July 22, 1908. 7, 8, and 9, behind Botany School. I in open bed in Botanic Garden, for comparison. Leaves 8 and 9 were in the shade of lime trees till 9 A.M.; Leaf 7 till 11 A.M.

same day is given for comparison. The chief point of interest is the contrast between Leaf 7 and Leaves 8 and 9 at 10.15 A.M. The shadow of an avenue of limes left the latter about 9 A.M., so that by 10.15 they had been in full sunlight for more than an hour. Leaf 7, on the other hand, belonged to a plant several yards nearer to the trees, and was not insolated till 11 A.M. Consequently by 10.15 the latter had decreased in area very little, only 0·5 per cent., while Leaves 8 and 9 show a decrease by the same time of 1·8 per cent.

Sachs' Experiments and Shrinkage Errors.

The extreme importance of shrinkage as a source of error in the dry-weight method is fully demonstrated by these results. To show the relative magnitude of the errors which are possible, it will be convenient to take the experiments described by Sachs and estimate the errors that shrinkage may have introduced into his results.

There is much difficulty in selecting, from my measurements of shrinkage, results which can fairly be compared with Sachs' experiments, for there is no means of knowing to what extent he took notice of differences in the appearance of the leaves he used. One hesitates to put a high value to the personal equation of an experimenter of Sachs' experience and ability; although, on the other hand, after an interval of 10 hours, slight differences are easily overlooked, and even half-consciously ignored when the extent of their effect on the experiment in hand is not suspected. What follows is therefore not to be regarded as finally condemning Sachs' original results, however much doubt it may throw on their trustworthiness.

He commenced his experiment with attached leaves of *Helianthus annuus** at 5 A.M. on August 13, 1883, by cutting seven half-leaves from one large plant. The other halves he took at 3 P.M., after 10 hours of clear sunny weather with blue sky. The day was warm; the highest temperature he records was 25° C. at 3 P.M.

It is quite certain that a shrinkage of 2 or 3 per cent. during this experiment would have been overlooked, while if Sachs used robust leaves, similar to Leaf II described above, he might easily have allowed a change of area of 4 or 5 per cent. to pass unnoticed.

Perhaps, however, it will be better to take a general average from actual observations. The following table contains my measurements made in the summer of 1908 under weather conditions which were in several cases less severe than those holding during Sachs' experiment:—

* *Loc. cit.*, p. 23.

Table XVII.

	Maximum temperature in degrees C.	Weather.	Average percentage decrease in area of Leaves I—IV between 5 A.M. and 3 P.M.
July 22	25·3	Clear	2·8
,, 24	26·6	Hazy	1·4
,, 26	24·4	Bright: white clouds.................	2·3
,, 27	23·8	Clear, changing to rather cloudy, then hazy	3·0
,, 30	27·6	Clear: some wind	5·6
August 3	22·2	Clear	7·3
	Average of all six days		3·7

Applying such a medium correction to Sachs' result, we obtain the following:—

	Sachs' results.	Results "corrected" for shrinkage.
	grammes.	grammes.
Dry weight of 700 sq. cm., 5 A.M............	3·054	3·054
3 P.M............	3·693	3·556
Gain 10 hours	0·639	0·502
Gain per square metre per hour	0·914	0·717

If 0·7 gramme is really the true result, Sachs' value was too high by 0·2 gramme, *i.e.* by about 30 per cent.

Brown and Morris, in their repetition of this experiment under similar weather conditions, terminated it two hours later than Sachs.* It is possible, therefore, that their smaller result is to be accounted for by the fact that their experimental half-leaves had had two hours longer in which to recover from shrinkage.†

Sachs' experiment with *Rheum*‡ is also liable to a great error. In this case he commenced at 6 A.M. and ended the experiment at 11 A.M. after five hours of prevailing sunshine. He obtained for the gain of dry weight per square metre per hour the value 0·652 gramme.

In one of my experiments, when the sunshine was only intermittent, four marks were made on a large Rhubarb leaf, roughly in the positions which would be occupied by the

* *Loc. cit.*, p. 627.

† It may not be entirely fortuitous that their result, 0·713 gramme per square metre per hour, agrees closely with the value 0·7 obtained above by introducing a correction for shrinkage into Sachs' result.

‡ *Loc. cit.*, p. 24.

four corners of a 100 sq. cm. templet laid upon the leaf. A decrease in the enclosed area of 2·5 per cent. was observed between 8 A.M. and 1.30 P.M. Such a change during Sachs' experiment would have meant an over-estimate of the gain per square metre per hour of 0·16 gramme, *i.e.* 33 per cent., the "corrected" result being 0·49 gramme.

The relative magnitude of the error introduced by a given change of area is greater the smaller the total photosynthetic increase, the shorter the time of experiment, and the greater the average dry weight of the particular leaves used. On each of these considerations Sachs' experiment with attached leaves of *Cucurbita pepo** is liable to have been vitiated by shrinkage errors to a far greater extent than those with *Helianthus* and *Rheum*.

The average dry weight per unit of area of the *Cucurbita* leaves was greater than that of his *Helianthus* leaves (56 grammes as compared with 44 grammes per square metre), and as the experiment lasted only three hours, the total increase to be expected was comparatively small, and any error was distributed over 3 hours instead of 10 as in the *Helianthus* experiment. In addition to this, the second halves were taken at noon, just the time when, as a rule, the shrinkage is most pronounced.

Sachs commenced his experiment at 9 A.M. on August 21, the temperature rising from 18° C. at that hour to 24° C. at noon, and sunshine continuing throughout. He found an increase of 0·68 gramme per square metre per hour.

Under similar (not extreme) conditions I have observed a shrinkage in area of 3·6 per cent. in a leaf of *Cucurbita*. A change of area by this amount would mean a corresponding apparent increase of 0·7 gramme per square metre per hour, so that *the whole of the observed difference might have been due to shrinkage.*

From these considerations it is clear that the results of all Sachs' experiments with leaves attached to the plant are open to doubt; they may all be too high to a greater or less degree.

Sachs' Experiment with Detached Leaves of Helianthus annuus.

The high result which Sachs obtained for *detached* leaves† suggests the possibility that here, too, shrinkage may have introduced a considerable error, especially as Brown and Morris's results for detached leaves‡ were much smaller. When, however, the details of the experiment are examined, this seems less probable.

Sachs cut eight leaves of *Helianthus annuus* at 5 A.M. and set them in water in the laboratory in dull light till 8 A.M. He exposed the experimental halves in the garden with their stalks in water from 8 A.M. till 2.45 P.M. Finding then that they were flaccid, he stopped the experiment and immersed them in water for half an hour to make them turgescent.

The following experiment shows that this treatment probably eliminated shrinkage :—

Three leaves were detached and left for an hour in a dull light with their stalks in water. After measurement of the distances between marks on the leaves, in the way

* *Loc. cit.*, p. 23.
† *Loc. cit.*, p. 25.
‡ *Loc. cit.*, p. 628.

already described,* the leaves were made flaccid by exposure to sun and wind, and the measurements repeated. At this stage Leaves 1 and 2 were very flaccid, Leaf 3 much less flaccid but drooping. They were then immersed in water and measured after a quarter of an hour, and again at the end of an hour's immersion. The measurements are given in the following table :—

Table XVIII.

Column (1) .... Leaf 1, transverse.
,, (2) .... ,, 1, along midrib.
,, (3) .... ,, 2, transverse, left half-leaf.
,, (4) .... ,, 2, ,, right ,,
,, (5) .... ,, 2, along midrib.
Columns (6), (7), (8)...... ,, 3, as for Leaf 2.

Numbers in centimetres.

	Leaf 1.		Leaf 2.			Leaf 3.		
	(1.)	(2.)	(3.)	(4.)	(5.)	(6.)	(7.)	(8.)
(*a*) Initial dimensions	14·51	16·33	9·40	12·50	9·30	8·87	8·76	13·97
(*b*) When flaccid	13·64	16·02	8·60	11·69	9·19	8·70	8·57	13·91
After immersion in water for—								
(*c*) $\frac{1}{4}$ hour	13·73	16·15	8·82	11·79	9·19	**8·92**	**8·82**	**14·01**
(*d*) 1 ,, ...	**14·58**	**16·38**	**9·47**	**12·48**	**9·28**	8·92	8·87	14·06

It will be observed that even Leaves 1 and 2, which had been so flaccid that Sachs would probably have rejected them, more than recovered in an hour's immersion. Leaf 3, which had been but moderately flaccid, recovered in a quarter of an hour. It is highly probable, therefore, that Sachs' experimental half-leaves were completely recovered after the half hour's immersion which he gave them. It follows that no deduction can be made from his result on the ground of a shrinkage error. After all possible deductions have been made it still remains substantially the same, still remarkably high.

It may be mentioned here that experiments have been carried out which support Sachs' result.† The full description of these experiments, and a discussion of their relation to Brown and Escombe's experiments, will be postponed till more evidence has been obtained.

Shrinkage Phenomena in General.

It remains to consider the phenomena of shrinkage as they appear in other plants than *Helianthus annuus*, and to discuss in a more general way their bearing on the dry-weight method.

* See p. 23.

† See Thoday, D., "On Increase in Dry Weight as a Measure of Assimilation," 'Report of the British Association, Dublin,' 1908, p. 905.

In the first place, to indicate how universal are the phenomena in greater or less degree, the following selection is given from measurements of a number of leaves of various types.

Table XIX.—Percentage Decrease in Area (estimated) of various Leaves during Insolation.

August 9, 1908, 6 A.M. to 12 noon. Bright warm day, occasional clouds.

Cercis, four leaves	1·6
	1·6
	2·4
	2·0
Nicotiana, two leaves	1·8
	4·4
Cucurbita	5·6*
Vitis	0·6
Cherry Laurel, three leaves	1·2
	1·2
	0·9

August 10, 1908, 8 A.M. to 1.30 P.M. Cloudy, occasional spells of sun.

Cucurbita, three leaves	{1·4, 1·0}
	{2·6, 2·6}
	2·5*
Rheum, two leaves	2·5
	1·6
Sugar Beet, three leaves	0·6
	1·7
	3·1
Saxifraga, sp. with large thick fleshy leaves	0·8

Shrinkage is shown by all the leaves examined, though in varying degree. Thus, while *Cucurbita* decreases in area by 5·6 per cent, *Vitis* decreases by but 0·6 per cent. Leaves of the same plant may also show considerable differences from one another (*Nicotiana*, Sugar Beet), as was found to be the case with *Helianthus annuus*.†

Xerophytic leaves show relatively little shrinkage (Cherry Laurel, *Vitis*, *Saxifraga*), partly owing to slower evaporation, partly to the mechanical resistance of cuticle and internal strengthening tissue to contraction. Where a leaf is also thick and fleshy (*Saxifraga*) or possesses tissue for water storage,

* These two results were given by the same leaf, and afford a relative estimate of the conditions which held on the two days.

† See p. 22, etc.

a considerable diminution in thickness might take place before much change of area would be effected.

A uniform close-meshed network of veins, such as occurs in *Cercis* (and in Lime), also resists shrinkage. In leaves which shrink most, on the other hand, the ultimate ramifications of the veins are more slender and delicate (*Nicotiana, Cucurbita*).

The conditions on August 9 were fairly severe, but *Vitis, Cercis,* and the Cherry Laurel gave to the eye no sign whatever of decreased turgidity. Yet the *Cercis* leaves had shrunk on the average nearly 2 per cent., and Cherry Laurel over 1 per cent. *Vitis* is the most resistant of all the leaves observed.

The conclusion from all the results is that shrinkage is a general phenomenon, and can occur to the extent of 2 or 3 per cent., or sometimes more, without very obvious signs of flaccidity accompanying it. It is, therefore, highly desirable from the point of view of the half-leaf dry weight method that shrinkage should be eliminated or corrected for.

Treatment of Detached Leaves.

Greater care to ensure turgidity during experiment might be useful in many cases; but this is difficult in the sun, as all observers have noted. The difficulty is especially great with detached leaves, and most of those who have employed them have worked either entirely in the shade or with translucent screens.

To modify the illumination is, however, not always admissible; but another factor, the water supply, is amenable to control. In cutting leaves from the plant, air is apt to enter and block the cut vessels, and so hinder the passage of water to the lamina.

Sachs' method of collecting his material in the early morning when there was little transpiration is the most efficient way of avoiding this.

Brown and Escombe, gathering their leaves later in the day, cut through the petioles under water.* In experimenting with *Helianthus tuberosus*, however, even leaves so treated were found to droop very rapidly in the sun. If, on the other hand, freshly boiled distilled water was used, they remained turgid much longer.

Steaming the cut end of the petiole is sometimes of advantage. Its success may be due either to the driving of air from the vessels or to the killing of the tissues and the surrounding of the vessels on all sides with available water. The treatment is objectionable, however, since there is danger of secondary injurious effects. Moreover, it is not always more successful than careful cutting under water.

It is probable that almost the whole difficulty in the treatment of detached leaves is due to the blocking of vessels by air: for in an experiment with *Helianthus annuus,* for which leaves were cut from the plants at 5.45 A.M., while still wet with dew, the use of freshly boiled distilled water was quite sufficient to ensure, in three leaves out of five, a degree of turgidity even greater than would have existed on the plant itself under similar conditions.

* *Loc. cit.*, p. 57.

The use of air-free water is undoubtedly an advantage. In the sun the leaf is heated to a high temperature, and, if well aerated water is used, air is set free in the vessels. Air-free water, on the other hand, may even dissolve any air bubbles that already exist in the water channels.

The water supply to attached leaves may be improved by watering the ground thoroughly. Even this might be inadmissible in œcological investigations, and there are obvious objections from the same point of view to shading the leaves from the full intensity of the sun. Even in investigating some questions of pure physiology shading is to be avoided; but for many purposes this plan could with advantage be adopted as an accessory means.

It is also possible, with attached leaves, to choose the time of the experiment in such a way as to ensure approximately the same degree of turgidity at the beginning and end of the experiment. This would often be difficult and uncertain, however, as under different weather conditions the duration of shrinkage and rate of recovery vary enormously.

Elimination of Shrinkage Errors.

We will now turn to questions of more immediate importance, and discuss methods of completely eliminating or correcting for change of area.

A. *Immersion in Water.*

The most obvious way of eliminating shrinkage errors is to bring the experimental half-leaf to its original condition of turgidity before determining the area.

The experiment with leaves of *Helianthus annuus* testing the effect of soaking them in water, and other similar experiments, suggested that care in avoiding flaccidity, combined with immersion of both control and experimental half-leaves for half an hour before cutting or measuring the area, would suffice to eliminate shrinkage errors sufficiently accurately for many purposes.

Other experiments have shown, however, that this is not always the case, and have revealed other difficulties. Even when both half-leaves are soaked, the experimental half-leaf after exposure to light may expand too much relative to the control half-leaf. With a detached leaf of *Dipsacus* sp. a difference of as much as 1·6 per cent. was observed. In an assimilation experiment with detached leaves of *Helianthus annuus* the excess varied between 0·9 and 1·2 per cent.

Such changes as these, of course, introduce a negative error into the uncorrected increase of dry weight, which, though small, is not negligible if the method is to be of much service in solving problems of interest to the pure physiologist.

Another difficulty has presented itself in my experience with *Dipsacus* leaves. The longitudinal dimensions near the midrib may be appreciably different when the midrib is still attached and after it has been removed. The change is fairly small as a rule, but for accurate work it is necessary to take care that the conditions under which the two half-leaves are measured are similar in respect of the tissue tensions existing, as well as of the state of turgidity. When the templet method is used it is sufficient, if the midrib be stout, to cut pieces from the control half-leaf before removing it, for the resistance of the midrib itself will prevent deformation of the experimental half-leaf owing to the relaxing of the tension on one side. Otherwise, the control half may be

left attached; but this is not always convenient, and is out of the question if it is desired to measure the whole of each half-leaf, by the planimeter method. However, under comparable conditions of turgidity the tissue tensions would probably be similar on the two sides, and if the midrib is removed from the experimental half-leaf before measuring it the error is likely to be reduced to a minimum. This was found to be the case in experiments with *Dipsacus;* the change in the longitudinal measurement of the control half-leaf following its separation from the rest of the leaf was approximately the same as the corresponding change in the experimental half-leaf consequent on the removal of the midrib.

To sum up, if care is taken to prevent the experimental half-leaf from becoming very flaccid, soaking both half-leaves in water eliminates large shrinkage errors; but a more certain and accurate method is desirable.

B. *Correction for Shrinkage.*

An alternative plan, which avoids the uncertainty that attaches to soaking, is to make a correction for shrinkage.

(1) The method of estimating this correction, from changes in linear dimensions, has been described already. Though only approximate it has been used successfully in several experiments. The marks should be placed symmetrically on the two half-leaves, and with reference to the portions to be used.

Measurement between marks on the leaf with a millimetre scale can only be applicable where the leaves used are large enough for the dimensions measured to approach 10 cm.

For small leaves similar methods could be adopted, based upon the use of a scale with a vernier, or of a small portable microscope with a micrometer eye-piece. The use of the microscope, which would involve the attachment of some form of index to the leaf, might prove somewhat clumsy, and would take longer than measurement by eye. Any form of measurement, indeed, must occupy a considerable time, and it would often be quite impossible to measure the large number of leaves necessary to reduce the asymmetry error within reasonable limits. On the other hand, for many purposes a sufficiently accurate estimate of the average change of dimensions could be obtained from measurements of a few representative leaves. I hope to give some further attention later to the question of the most convenient apparatus and technique for this purpose.

(2) The method adopted by Brown and Escombe for measuring the shrinkage of *Catalpa* leaves is also available as a general method for estimating the shrinkage correction. They took prints of half-leaves still attached to the midrib and petiole before and after exposing them to their experimental conditions, and measured these prints with a planimeter. By following this plan a measurement of the degree of alteration in area would be obtained directly, and, subject to the limits of accuracy of measurement by planimeter, would be more satisfactory than the estimation of area changes from changes in the linear dimensions of selected regions of the leaf. It would be less suitable for attached than for detached leaves, since to obtain a print of a leaf while still on the plant would be an awkward process.

C. *The Stamping Method.*

The simplest and most satisfactory method of eliminating all possible errors from change of area is to mark out the area in some way at the very

beginning of an experiment. The stamping method described in Section VI has been devised to fulfil this requirement. The principle of the method is to stamp each half of a leaf with a rectangle, by means of a specially constructed rubber stamp, and to cut out with scissors the area so delimited.

This method, considered merely as a means of determining area, is with care probably as accurate as Sachs' templet method, and for many plants must therefore supersede the latter. Further discussion of this new method will be found in Section VI.

In conclusion, it may be said without hesitation that errors from change of area during experiment are by far the most serious and most difficult to deal with of all the errors to which the Sachs dry-weight method in its original form is liable. In consequence of the ignoring of these errors most of the results which have hitherto been obtained with the method require revision and repetition.

The difficulties are not, however, insurmountable. Changes of area can be estimated and corrected for, although the technique involved must be laborious. On the other hand, the stamping method of area determination, by the use of which shrinkage errors are eliminated, is almost as simple in use as Sachs' templet method, and it is hoped will prove widely applicable.

Note.—Shrinkage errors affect any method which involves measuring the difference between the amounts of a substance contained at different times or under different conditions in unit area of leaf surface. The error is relatively great only when the fluctuations are of a lower order of magnitude than the average content. Thus, when increase in proteid content is to be measured, shrinkage errors might be almost as serious as they are in measuring increase of dry weight : on the other hand, they could probably be ignored in determining changes in the amounts of starch and other carbohydrates.

A case which is probably an illustration of this difference in relative magnitude of shrinkage errors is to be found in Menze's comparison of increase of dry weight with gain of carbohydrates (*loc. cit.*, p. 37). Although the conditions under which he conducted his experiments were not such as would tend to produce large area changes, his ash determinations favour the assumption that some area changes did occur : if so, an appreciable part of the excess increase of dry weight which is unexplained by the gain of carbohydrates might be only apparent. On the other hand, some of the surplus probably represents proteid formed, and the fluctuations in ash content may have been in part or wholly real (*cf.* Section III, p. 9, footnote *).

A better illustration is afforded by Saposchnikoff's results (*loc. cit.*, see p. 10, *et seq.*). He estimated proteids, as well as starch and soluble carbohydrates : nevertheless, the increase of dry weight was still not completely accounted for, and he fell back on cellulose as the possible form of the unexplained excess. It is much more probable that most of the excess represents apparent increase of dry weight due to shrinkage. The high rates of increase indicated by some of his shorter experiments favour this interpretation.

Section VI.—On the Measurement of Area.

The only important methods hitherto used for determining the area of the portion of leaf of which the dry weight is required are Sachs' templet method, and the planimeter method, used by Brown and Escombe, consisting in the measurement of a photographic print by means of a planimeter.

Two new methods have been devised in the course of this research. One is the punch method, a modification of the templet method depending upon the use of a rotating punch; the other is the stamping method, by which shrinkage errors are eliminated.

The Templet Method.

In this method a piece is cut from the leaf of the same area as a rectangular plate of wood, metal, or glass laid upon it. This is by far the simplest and most rapid method.

Sachs gave the error involved in his use of the templet as a few square millimetres in an area of 50 or 100 square centimetres, or only a few ten-thousandth parts of the whole.* This must, however, be taken as a rough estimate.

Müller† states that in cutting an area of 40 sq. cm. with a templet the limit of the error introduced into the dry weight per square metre varies from 0·25 to 0·66 gramme, according to the prominence of the veins. This is of the order of 1·5 per cent. and much too high. Since he does not say how he arrived at the estimate, no weight can be attached to it.

There is no perfectly satisfactory method of measuring the degree of accuracy of the templet method directly. For instance, to cut out a given area from paper with a templet and then measure it accurately would only give a minimum value for the error involved in cutting a similar piece from a leaf. The leaf is held firmly only at the veins, and it is difficult to avoid some small displacement of the extensible tissue which intervenes.

To obtain a *maximum* estimate of the error, pieces were cut in the usual way from leaves, and photographic prints of these pieces taken immediately on "gelatino-chloride" paper. The untoned prints were subsequently measured by means of a scale of centimetres with a vernier reading to 0·1 mm. A number of measurements were made of the longitudinal and transverse linear dimensions, and from these the area of the print was estimated.

Before discussing the results obtained in this way it is necessary to remark that the only point of immediate importance is the degree of concordance between results obtained with similar leaves. The area of the pieces at the time of printing was likely to be different from their area when the templet was laid upon them and the cuts made, owing to freedom from tension and some loss of water by evaporation. It is, therefore, in accordance with expectation that the areas of leaf pieces given below are all less than the area of the templet used in cutting them; and also that this absolute error is greater for *Helianthus annuus*, which has been shown to shrink so rapidly.

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* *Loc. cit.*

† "Die Assimilationsgrösse bei Zucker- und Stärkeblättern," 'Jahrb. f. wiss. Bot.,' vol. 40, 1904, p. 443.

Accordingly the results for each kind of leaf will be considered separately. They are briefly set forth below.

Two templets were employed, measuring respectively 8 cm. by 5 cm., and 4 cm. by 5 cm. When accurately measured, their areas were found to be 40·2 sq. cm. and 20·1 sq. cm.

Bucklandia populnea: leaves smooth and leathery, with prominent principal veins.

(a) *Templet*, 40·2 *sq. cm.*

40·1 sq. cm. } Rectangles cut from the same leaf and printed together in the
40·0 „ } same frame.
*39·9 sq. cm. } Rectangles cut from the same leaf and printed together in the
39·8 „ } same frame.

Maximum difference from the average ±0·15 sq. cm., *i.e.* ±0·4 per cent.

(b) *Templet*, 20·1 *sq. cm.*

20·0 sq. cm. } Printed together.
19·9 „ }
20·0 sq. cm. } „
19·9 „ }

Maximum difference from the average ±0·05 sq. cm., *i.e.* ±0·3 per cent.

Omalanthus Leschenaultianus: leaves softer than those of Bucklandia, but smooth and fairly firm, with prominent principal veins.

Templet, 40·2 *sq. cm.*

40·1 sq. cm. } Printed together.
40·1 „ }

Difference, nil.

Ranunculus sp.*:* leaves large, with soft irregular lamina between a prominent network of veins; specially selected as *unsuitable* for use with templets. The pieces were cut and printed separately.

Templet, 40·2 *sq. cm.*

40·2 sq. cm.
40·1 „
40·0 „

Maximum difference from the average ±0·1 sq. cm., *i.e.* ±0·3 per cent.

Templet, 20·1 *sq. cm.*

19·9 sq. cm.
19·9 „

Difference, nil.

Helianthus annuus: leaves large, fairly thin, with minor veins somewhat outstanding, but otherwise lamina fairly flat. Printed in the summer; this fact and the rapid shrinkage of the leaf account for the low average area of the prints.

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* This was a younger leaf than the first, and it is quite conceivable that it should be considered separately, on the same grounds as with leaves of different species. If so, the maximum difference is under ±0·2 per cent.

Templet, 20·1 *sq. cm.*

19·7 sq. cm.	Rectangles printed together in same frame.
19·5 „	
19·6 „	
19·6 „	

Maximum difference from average ±0·1 sq. cm., *i.e.* ±0·5 per cent.

The greatest error revealed here is 0·5 per cent.; and even this is likely to be an excessive estimate of the errors of the templet method itself, since changes of area from decrease of tension and evaporation of water may vary somewhat from piece to piece. The estimate thus includes errors in the method adopted to test the templet method, as well as the real errors of the latter.

It may be concluded, therefore, that the *greatest error* involved in cutting pieces from leaves *with templets as small as* 40 *and* 20 *sq. cm.* is *less than* 0·5 *per cent. of that area;* and in comparing two individual pieces the *maximum* error is less than 1 per cent. Such errors, therefore, only account for a small part of the differences measured in asymmetry tests, which reach maxima of 4 per cent. or more.

The magnitude of the error may be expected to vary with the character of the venation. If outstanding veins are excluded altogether, the error from extensibility is reduced to a minimum. The only other sources of error are the sloping of the cutting instrument, and inaccuracy in following the edge of the templet: such errors are reducible with careful cutting within very small limits. All the errors depend upon the perimeter, and so are less in proportion for larger areas.

Where outstanding veins were few, templets as small as 10 sq. cm. have been used, and the results of the asymmetry tests with *Paulownia imperialis** warrant the inference that the use of larger templets may be a distinct disadvantage from the point of view of asymmetry if veins are included that are at all prominent. These asymmetry determinations include the errors of measurement of area, but the differences are far too great to be accounted for by these errors, and it may safely be concluded that any possible disadvantage from the relatively greater perimeter, when small templets are used, is not to be compared with the advantage of reducing asymmetry by avoiding veins.†

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* Pp. 17 and 18.

† For small leaves, Müller felt that the chance of the cutting error becoming serious if small templets were employed made some other method desirable (*loc. cit.*). He took sunprints of half-leaves, and estimated the area by cutting out the print and comparing its weight with the weight of a known area of the same paper. He found that when equal areas were cut out of different sheets from the same packet, the maximum error was equivalent to 0·52 sq. cm. in an area of 40 sq. cm. This is equivalent to 1·3 per cent. of the total area. It would seem, therefore, even taking his own estimate of the maximum error in the templet method, 1·5 per cent., that the only advantage of his printing method lies in the use of entire half-leaves, instead of smaller areas cut from them; but his estimate is excessive, and the use of small templets is in reality much more accurate.

It would be easy to devise improvements of the templet method which would ensure very accurate cutting. By using thick templets with vertical edges and a cutting instrument with flat blade fixed vertically, capable of being brought down simultaneously on to the whole length of the required cut, all the errors that have been mentioned would be practically abolished. By such an instrument, too, a number of leaves could be manipulated at the same time without any sacrifice of accuracy.

It may be said in conclusion, therefore, that the errors of the templet method, though appreciable when the method is used in its original form, are capable of almost indefinite reduction.

The Rotating Punch.

A modification of the templet method has already been devised for the purpose of cutting small pieces from between veins, while at the same time avoiding the objections to using small templets by ensuring extreme accuracy in cutting. For the details of the construction of the instrument, Dr. F. F. Blackman was responsible. It consists of an adapted watchmaker's drill fitted with a circular cutter revolving on its own axis, and accurately turned *in situ* for this purpose. The cutting tube slides freely in a vertical direction, and the method of procedure is to set it in motion by means of a water turbine and then bring it down on the leaf, so cutting out a disc equal in area to that enclosed by the cutting edge. This can be repeated rapidly, a number of discs accumulating in the interior of the tube. A lateral slit facilitates their subsequent removal.

The Planimeter Method.

The planimeter method was that adopted by Brown and Escombe as a general method.

They estimate the error in their measurement of leaf-prints by a planimeter as "well under 0·1 per cent."* This is apparently to be taken as holding for areas as small as 40 sq. cm.,† although usually the areas measured were of the order of 100 sq. cm. The absolute error corresponding to their estimate must therefore be well under 0·05 sq. cm.

Where this method was adopted in the present investigation, the instrument used read by a vernier to 0·1 sq. cm. Under favourable circumstances, the maximum error found, due to inaccuracy in following the bounding line, was ± 0·1 sq. cm. in measuring an area of 27·5 sq. cm.‡ I am therefore inclined to regard Brown and Escombe's estimate as too low.

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* *Loc. cit.*, p. 59.

† *Cf. loc. cit.*, p. 60, Table IX.

‡ Besides this error others were found: on testing the instrument the graduation proved inaccurate, and in addition different results were obtained according to the average angle between the arms. It is thus very necessary to test a planimeter carefully before

There can be no doubt, however, that with a carefully tested and thoroughly trustworthy planimeter, reading to the nearest 0·1 sq. cm., this method would be convenient as a general method, and might be used for pieces cut from between the prominent veins of fairly large leaves without the error becoming prohibitive. In this way it would have the advantage over the templet method, that the form of the piece cut out would not be restricted, and a more complete use of the lamina would be possible while still avoiding the veins.

On the other hand, when describing the results which were obtained in testing the accuracy of the templet method, it was pointed out that the area of the prints was less than the original area of the pieces printed, owing to changes of tension and to shrinkage.* The magnitude of such changes will be less for entire half-leaves than for cut pieces; but in any case it is necessary to ensure as far as possible that the conditions of tension in portions to be compared are the same, during printing, and to adopt exactly the same technique, so that the times elapsing between the removal of the half-leaf, or portion, and the finished print are not only as short as possible, but equal. So long as the errors affect the measurement of control and experimental half-leaves in the same degree the absolute error can be ignored, since its effect in the calculation of the result for the unit of area is of the second order of magnitude.

Besides these errors arising from the varying condition of the leaves themselves, there are others which may be called purely mechanical.

The leaves may not be perfectly smooth and level, and the pressure to which they are subjected during printing may vary. The former difficulty is frequently encountered with leaves like *Tropæolum*, which are sometimes waved at the edges, and in such a case it was found necessary to cut triangular pieces from the waved parts, so as to make them lie flat. This is objectionable, both because of the danger of multiplying the circumference error, and from the increased number of planimeter measurements involved in the separate printing of the detached pieces. It is impossible always to exclude leaves which show this waving of the edge, for under the initial conditions of turgidity it may not be shown at all.

If the same printing frame is used for both halves of a leaf, variations in pressure are scarcely possible to an appreciable extent.

The effect of varying pressure is more likely to be of account in the templet method: but on general grounds, and from a consideration of the results of the test described above, it can be assumed that when a leaf is flat the alteration of area caused by pressure of the templet upon the leaf is very small, even if it is at all appreciable; and if the leaf is not flat the error is insignificant in comparison with that resulting from asymmetry. A leaf of *Catalpa* was tested, in which one side was bulged between the veins, while the other side was flat. Pieces cut by the same templet from each side differed in dry weight to the very exceptional extent of 8 per cent. in favour of the bulged side, whereas the average degree of asymmetry for *Catalpa* is ± 3·5 per cent. Such errors as these can be

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using it for accurate work. To distinguish between errors due to lack of skill in following the perimeter and those due to inaccurate graduation, it is sufficient to take a number of successive readings in a clockwise direction, and then to traverse the same part of the scale again in the counter-clockwise direction. By repeating this several times, a number of readings are obtained in each of several successive parts of the scale.

The planimeter was only used in preparing the material for the earliest analyses (Table I, p. 6), and since eight readings were averaged, the resulting error was insignificant.

* Pp. 39 and 40.

avoided by careful selection of the leaves to be used for experiment, and are, therefore, not inherent in the method.

It may be remarked that by cutting small areas, as with the rotating punch described above, the error from slight bulging would be considerably less than when a large templet is used.

The Stamping Method.

The new method, which has been devised to eliminate shrinkage errors, depends upon marking out a known area on each half-leaf before experiment.

Preliminary tests have been carried out with an inked rubber stamp, making an impression of a rectangle measuring 5 cm. by 2 cm. The areas so marked out were cut from the leaf with scissors. To test the accuracy with which this can be done, a number of impressions were made on a piece of paper, cut out, and measured by means of a scale with a vernier reading to 0·1 mm.

In cutting, the outside of the line was followed, and hence a positive difference from the actual area of the rectangle marked out was always found, but the degree of uniformity which can be assured by a constant procedure is the question of most interest and importance.

The results were as follows:—

Area marked out.	Areas found after cutting out.
10·94 sq. cm.	11·09 sq. cm.
	11·07 "
	11·09 "
	11·11 "
	11·04 "
	11·10 "
Average	11·08 "

The difference between the extreme values is here 0·6 per cent. of the total area. Considering the smallness of the area, this compares very favourably with the templet errors as estimated above.*

Similar tests have not been applied directly, using leaves, as in doing so the same difficulties present themselves as were met with in testing the templet method. Experience with Cherry Laurel, however, has shown that to follow a line with scissors is easier with a soft leaf than with tougher paper; and it is believed that, especially when larger areas are used, this method will be quite as accurate as, if not more accurate than, the templet method. It will be employed in some projected experiments with the dry-weight method by which the solution of some of the more important problems of assimilation in the open air will be attempted.

* Pp. 39—41.

Section VII.—On the Killing and Drying of Leaf Material.

In view of Brown and Escombe's suggestion that the colloids of the leaf may undergo an alteration in their power to retain water, it was essential that special care should be taken to ensure the complete drying of material to be analysed by combustion.

Brown and Escombe dried their *Catalpa* leaves at 100° C. in a current of dry air or dry hydrogen. As dry leaf material is extraordinarily hygroscopic, I have used a modification of this method in which all contact with the outer air is avoided until the material has been weighed.

The pieces of leaf were first killed by steam as Sachs recommended,* then dried roughly in a water oven; and, finally, dried at 100° C. in a current of dry air in a special glass apparatus enclosed in a steam bath.†

The requirements for subsequent analysis made it desirable to use comparatively small quantities of material; therefore, to avoid the relatively great loss which powdering would entail and the trouble of estimating it, the material was so treated that it would easily fit the porcelain boat while remaining intact.

This was done in two ways. In using the templet method the pieces of leaf, after being cut out, were rolled up into cylindrical form, retained in this form by a spiral of platinum wire, and killed by suspension in steam. In the process of killing, the roll shrank in diameter and became flaccid, and could be removed with ease, without fear of its becoming unrolled. It was then dried in a drying oven, on a curved metal support. Care was taken to put the free edge below, and this precaution, aided by the curvature of the support, prevented the roll from curling out of shape in drying.

The other method of preparation was connected with the use of the rotating punch. The circular cutting edge had a diameter of about 1 cm., and the discs so cut from one half-leaf were threaded on a thin weighed glass needle. After killing, by suspension in steam, a number of such needles of material were put into the ordinary drying oven in special supports made by sticking a number of short narrow pieces of glass tube radially into a bung, the needles fitting into the radiating tubes.

The apparatus in which the final drying took place (see fig. 8) was of such a shape and size as would conveniently accommodate the porcelain boat in which the material was to be burned, so that it could be dried finally in the boat immediately before analysis. The air was heated, before entering the wide tube (B) in which the leaf material was placed, by passing through a narrow tube (A) sealed to the wide tube and spirally coiled round it. Both tubes passed through the same cork (C), which supported them in a specially constructed steam bath (G). The whole formed a convenient and compact piece of

* There is a theoretical objection to putting pieces of leaf straight into the oven in which the preliminary drying is to take place, for the rate at which the material is heated up is relatively slow, and respiration, which increases greatly with the temperature, must entail some loss. The error is probably small, especially when the portions to be compared receive identical treatment. On the other hand, the rapidity with which steam kills the material, owing to its high latent heat, ensures the reduction of all such errors to a minimum. This method has been adopted for all except a few early experiments, and identical treatment given in every respect to portions which were to be compared.

† Heating to 108° C. in a toluol bath drove off no more water.

apparatus. To avoid contact with the outer air between drying and weighing, the material was weighed in a tube which fitted into the open end of the wide drying tube (D); the joint was made fast by a short length of wide rubber tubing (E), and air, dried by passing through tubes containing calcium chloride and sulphuric acid, was drawn through the whole by means of a water pump with a water resistance to keep the suction force steady. After drying, the material was tilted back into the weighing tube, which was then removed, closed at both ends, and left to cool in a desiccator. When cool, the tube was opened for a moment to equalise the pressure within and without, and weighed closed, and again weighed after removing the material.

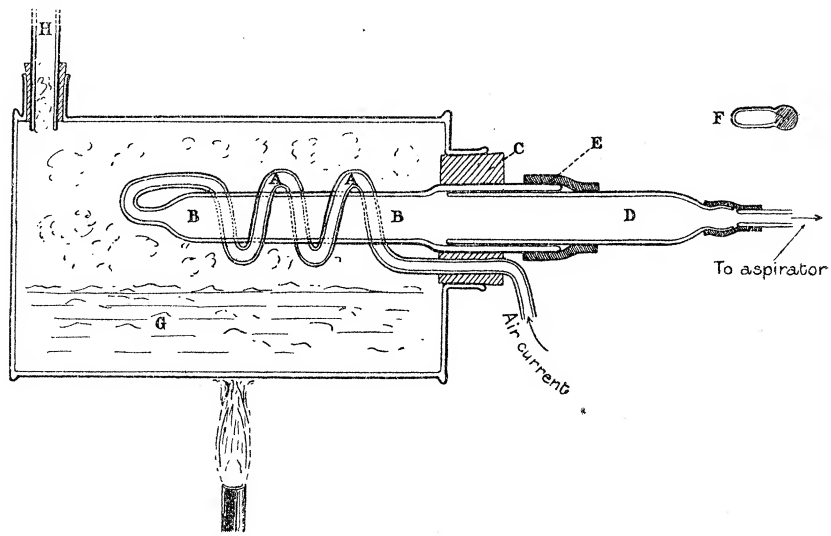

Fig. 8.—A, spiral tube in which air is heated in passing to B, the wide tube in which leaf material is placed to be dried. A and B pass through same cork, C. D, weighing tube, fitting into wider open end of B. E, short length of wide rubber tubing, making D fast to B. F, glass stopper for small end of D; the other end is closed by a rubber stopper. G, cylindrical steam bath. H, glass tube functioning as condenser.

By this method 0·1 to 0·3 gramme of leaf material was often dried and weighed repeatedly without the successive weights obtained varying by more than one-fifth of a milligramme.

After the material had been dried roughly in an ordinary water oven, it was found sufficient for most purposes, for instance in investigating the asymmetry of leaves, to pass a moderate current of air for 15 to 30 minutes before weighing; but the material analysed by combustion was always very carefully dried, usually for an hour at first, and then two or three times for periods of half an hour to ensure absolute constancy of weight.

A really satisfactory method of drying having been devised, attention was turned to the methods which were used by Sachs and others.

Some comparisons have been made of the results obtained by drying in an ordinary water oven and weighing in glass weighing bottles, with the results found after drying at 100° C. in a current of dry air. In making them it was found to be impossible to dry to a constant weight in the oven, and the portions of material from two halves of a leaf were, instead, dried together for the same length of time and treated as nearly as possible in the same way in every respect.

The results of four such experiments are given in the following table. The materials used in two of these were obtained from assimilation experiments with *Tropæolum majus*, in the other two from translocation experiments with *Helianthus tuberosus*.

The areas in the experiments with *Tropæolum* were measured by the planimeter method. Any error entering into the determination of the area does not, of course, affect the comparison.

Table XX.—Comparison between results obtained with a drying oven and with a current of dry air at 100° C.

Weights in grammes per square decimetre.

	Area, sq. cm.	Oven.		Air current.	
		Dry weight.	Difference.	Dry weight.	Difference.
Tropæolum majus I (a) ……	46·2	0·2063	+0·0116	0·1978	+0·0110
(b) ……	41·8	0·2179		0·2088	
" " II (a) ……	40·9	0·2303	+0·0019	0·2220	+0·0021
(b) ……	44·9	0·2322		0·2241	
Helianthus tuberosus I (a) …	60	0·4541	−0·0430	0·4448	−0·0440
(b) …	60	0·4111		0·4008	
" " II (a) …	50	0·4400	−0·0334	0·4300	−0·0340
(b) …	50	0·4066		0·3960	

These results show that although after drying in the oven a further 4 per cent. of water may be driven off when the material is dried in the current of dry air at 100° C., yet the difference in the values for the gain of dry weight is small. The greatest difference is 0·001 gramme per square decimetre, which is less than 0·2 per cent. of the total dry weight.

Considering the magnitude of the errors from other sources, this is a fair degree of approximation, so that the water-oven method of drying is permissible for rough experiments if care is taken to treat in exactly the same way the two portions of material to be compared, and, preferably, to give them their final drying together. It is not to be recommended if a current of dry air or other gas can be used.

The apparatus described here is not adapted either in form or size for very general use, although it has served the present purposes admirably. When a comparatively large quantity of material from a number of leaves is to be dealt with, a modification of Broocks' two-necked bottle ("Liebig'sche Ente")* would probably be more suitable, the current of dry air passing in at one opening, and out at the other, over the leaf material cut up into pieces of moderate size.

Two other points in Broocks' method are worth noting. He used a current of dry coal gas, and a temperature of 115° obtained with a paraffin bath. The high temperature, although unnecessary, probably increases the rapidity of drying, and the gas current could be used with great economy by passing it, carefully dried, over the material on its way to the burner.

* *Loc. cit.*, p. 16.

Section VIII.—General Considerations on the Degree of Accuracy and the Utility of the Method.

An attempt will be made in this section to estimate the degree of accuracy which can be obtained with the dry-weight method, and its probable utility in the future.

Since our knowledge of the composition of the true photosynthetic increase of dry weight is at present scanty, attention will be confined to the question *how accurately the gain of dry weight itself can be measured by the half-leaf method.* For this purpose it is only necessary to consider the errors due to asymmetry and change of area, as errors of technique are reducible within very narrow limits and are always included in measurements of asymmetry.

It will be interesting to begin by supposing that in an experiment lasting 5 hours it is desired to measure the increase of dry weight per hour per square decimetre correct to the nearest milligramme, a degree of accuracy which would be sufficient to allow a great deal of work to be done by the method;* and to consider the means necessary to secure this degree of accuracy.

For results correct to the nearest milligramme the total error in the increase per hour must not exceed $\pm 0 \cdot 5$ milligramme. If the experiment last 5 hours, the error in the total increase of dry weight during that time must be within $\pm 2 \cdot 5$ milligrammes per square decimetre.

The percentage error to which this limit corresponds will vary with the average dry weight per square decimetre of the leaf. This is widely different for different plants, as the following figures show:

Average Dry Weight of 1 sq. decimetre of various Leaves.

Alliaria officinalis	0·18	gramme.†
Helianthus annuus	0·33	,, †
Rumex sp.	0·41	,, †
Catalpa bignonioides	0·45	,,
Paulownia imperialis	0·6	,,
Cherry Laurel	1·0	,,

It may be instructive to calculate the desired limits of the probable percentage errors for *Helianthus* and the Cherry Laurel, as examples of two

* Brown and Escombe (*loc. cit.*, p. 61) infer from their own results with *Catalpa bignonioides* that a total error from asymmetry and shrinkage of ± 2 per cent. of the total dry weight is easily possible, which, in a 5-hour experiment, would mean an error in the increase per square decimetre per hour of ± 2 milligrammes; this is equal to the average rate of assimilation found by them for leaves of this plant by measuring the actual intake of carbon dioxide from ordinary air.

As a representative illustration this is unsatisfactory. *Catalpa* leaves are less symmetrical than the average, and there can be no doubt that a rate of assimilation of only 2 milligrammes per hour is greatly exceeded by many leaves in the open air (*cf.* Thoday, D. 'Brit. Assoc. Report, Dublin,' 1908).

† Müller, *loc. cit.*, p. 474.

very different types, the latter somewhat extreme. With the former an error of $\pm 2{\cdot}5$ milligrammes is $\pm 0{\cdot}8$ per cent. of the dry weight of a square decimetre. With the latter it is $\pm 0{\cdot}3$ per cent.

When the stamping method is employed, whereby shrinkage errors are entirely eliminated, the asymmetry error alone remains. In this case, the maximum error from asymmetry must be less than $\pm 0{\cdot}8$ per cent. of the total dry weight for *Helianthus*, less than $\pm 0{\cdot}3$ per cent. for Cherry Laurel, and the *probable* error not greater than about $\pm 0{\cdot}5$ per cent. and $\pm 0{\cdot}2$ per cent. respectively.

The average degree of asymmetry to be expected in individual leaves is about ± 2 per cent. Apart from the possibility of reducing asymmetry errors by avoiding prominent ribs, for which some experimental evidence has been given, the other means of reducing them is to use a large number of leaves. The probable error is thus reduced in the inverse ratio of the square of the number of leaves used. To bring the average error from asymmetry within the required limits, 16 leaves of *Helianthus*, or 100 leaves of Cherry Laurel, would therefore be required.

There are obvious practical objections to using a very large number of leaves, since the time taken in cutting out stamped areas with scissors is a consideration of real importance; as also in cutting out pieces by means of templets, or in taking photographic prints.

Moreover, with large-leaved plants like *Helianthus annuus*, bearing comparatively few leaves of which but a small proportion are perfectly sound and flat, the necessity of using such a large number of leaves would be a great drawback.

One hundred leaves of Cherry Laurel, too, would be quite unmanageable by the stamping method. On the other hand, with an improved templet or the rotating punch, it would be possible to manipulate a number of these fairly firm smooth leaves at the same time. With such leaves, which as a rule also show but a small degree of shrinkage under insolation, these methods are to be recommended in preference to the stamping method, a correction for area changes being estimated from measurement of representative leaves.

The accuracy with which shrinkage can be allowed for by measurement can only be roughly gauged. The error remaining after approximate correction is probably well within $\pm 0{\cdot}5$ per cent. of the total area, and therefore less than one-fifth of that due to asymmetry.

The conclusion seems unavoidable, nevertheless, that the probable error from all sources can in general only with difficulty be reduced, in a five-hour experiment, to so low a figure as $\pm 0{\cdot}5$ milligramme per square decimetre per hour. To attain to this degree of accuracy without making the method

too laborious, *the time of experiment must be increased, or the average degree of asymmetry for individual leaves reduced by avoiding veins.*

Thus if the average degree of asymmetry were for *Helianthus annuus* reduced to 1·5 per cent., that is by $\frac{3}{4}$, and the time of experiment increased to 10 hours, the number of leaves required, for the same degree of accuracy which demanded 16 leaves under the conditions originally assumed, would be reduced to $16 \times (\frac{3}{4} \times \frac{5}{10})^2$; that is, to two or three.

The number of leaves of *Helianthus annuus* used by Sachs for each experiment was seven or eight, and the same number gave Brown and Morris's difference of 1·1 per cent., which for a 10 hours' experiment works out to 0·4 milligramme per hour, and thus within the ± 0·5 milligramme error for which we have been calculating.

It will be convenient in this place to examine Brown and Escombe's experiments, in which they compared the apparent increase of dry weight, given by the simple half-leaf method, with the weight of carbohydrate corresponding to the carbon dioxide actually absorbed.

Considerations will be adduced in favour of the conclusion that the great discrepancies which they found are adequately explained by the asymmetry of leaves of *Catalpa bignonioides,* and their shrinkage under the experimental conditions.

Changes in ash content and inadequacy of the "carbohydrate factor" could account for a small part, but their effect may be ignored for the present purpose.

Before considering the results of the experiments themselves, it is necessary to estimate the probable magnitude of the errors. Unfortunately, this can only be done in a general way, as Brown and Escombe give neither the conditions under which the individual experiments were performed nor the time during which each lasted.

An instance of the possible *error from asymmetry* can be derived from the results of their own asymmetry test.* In each of their dry weight experiments they used four leaves, taking as control half-leaves the left and right halves alternately. Treating in this way the first four examples of *Catalpa bignonioides* from their asymmetry table, a difference is obtained, when the four leaves are taken together, of 2·1 per cent. The average dry weight per square decimetre of their *Catalpa* leaves is about 0·45 gramme, so that this error would have meant an under- or over-estimate of the total increase of dry weight by 9·4 milligrammes per square decimetre.

The possible errors from *shrinkage* must be deduced from the one experiment† in which Brown and Escombe measured the changes of area taking place in the experimental chamber used in their tests. They found the

* Quoted on p. 13.

† *Loc. cit.*, p. 60.

following percentage differences between the areas of the same half-leaves before and after exposure in the chamber:—

	Per cent.
(1)	−3·12
(2)	+0·98
(3)	+0·36
(4)	+0·14
Average ...	±1·1

The conditions under which these figures were obtained may have differed from those under which the actual assimilation experiments were performed. The leaves for the latter were covered, overnight, with tinfoil, in order to render them starch free. On the other hand, no mention is made of the like treatment of the leaves for this experiment on change of area. If this precaution were really omitted, the results may require a negative correction owing to the less turgid condition of the leaves when first enclosed.

Apart from this, the fact that one leaf had shrunk by as much as 3 per cent. shows that an average difference much greater than 1·1 per cent. might have occurred in some of their experiments. It is interesting in this connection that leaves of *Catalpa bignonioides* were found by Halsted* to shrink to an exceptional extent when dried for the herbarium; and I have observed that leaves of this species show very little sign of flaccidity, even after a shrinkage in area of 4 per cent.

Calculating first from their average change of area of 1·1 per cent., we obtain a possible positive error of 4·9 milligrammes per square decimetre in the total gain of dry weight. An average shrinkage of 3 per cent. would mean an error of 13·5 milligrammes. Adding the asymmetry error, assuming it to be positive, the total positive error becomes 14·3 or 22·9 milligrammes, according to the degree of shrinkage assumed.

This has to be divided by the number of hours, which is not stated Considering, nevertheless, that one of their dry weight experiments† lasted only 3¼ hours, we may perhaps calculate the error per hour on the basis of a 3-hour experiment, as well as of the 5-hour experiment for which their own calculation was made.

* Halsted, "Shrinkage of Leaves in Drying," 'Bull. Torrey Bot. Club.,' 1894, p. 129.

† *Loc. cit.*, p. 56, Table VII.

The respective errors in the gain per hour per square decimetre are as follows :—

Duration of experiment.	Error, assuming shrinkage to the extent of	
	1 ·1 per cent.	3 per cent.
	milligrammes.	milligrammes.
5 hours	2 ·9	4 ·6
3 ,, ...	4 ·8	7 ·6

Brown and Escombe's results, with which these estimates are to be compared, may now be given :—*

	Increase in milligrammes per square decimetre per hour.		
	Found by dry weight method.	Calculated from intake of CO_2.	Difference.
Experiment 1	9 ·8	1 ·8	+8 ·0
,, 2	7 ·1	1 ·8	+5 ·3
,, 3	2 ·6	2 ·9	−0 ·3
,, 4	7 ·2	2 ·9	+4 ·3
,, 5	Loss of weight.	3 ·0	Greater than −3 ·0

Since the greatest discrepancy between Brown and Escombe's gasometric and dry weight results is 8 milligrammes per square decimetre per hour, it is probable that *the very large and very variable errors found by them in the dry weight method are all explainable as due to asymmetry and shrinkage.* These will explain negative errors as well as positive : for asymmetry may have a positive or negative effect on the result ; and, as their own experiment shows, changes of area may be positive as well as negative, although the fact that they are in general more often negative accounts for the usual positive direction of the resultant error.

NOTE.—One other fact may be adduced in support of the explanation of these large errors by the occurrence of considerable shrinkage. In Table VII† they give three experiments with *Catalpa bignonioides*, lasting respectively 7, 5, and 3¼ hours.

The results per square decimetre per hour were as follows :—

In 7 hours	7 ·9	milligrammes
5 ,, ...	13 ·7	,,
3¼ ,, ...	16 ·6	,,

* *Loc. cit.*, p. 58, Table VIII.

† *Loc. cit.*, p. 56.

They show a greater increase the shorter the time of experiment, which is just the apparent effect that the division of a positive error over a varying number of hours would produce. The point cannot, however, be pressed, and the fact may have some other explanation ; but it is worth while to mention the possibility, especially as the results are so much higher even than those obtained in the experiments already discussed.

Section IX.—Conclusions.

1. Carbon analyses have shown that, except for minor differences, the same result is obtained for the rate of assimilation by the half-leaf method, whether increase of dry weight or of carbon content is measured. The dry weight method is therefore not vitiated by any large indeterminable errors such as would arise if varying quantities of water were retained by the colloids of the leaf after drying it at 100° C.

The calculation of the equivalent intake of carbon dioxide from the increase of dry weight can, however, only be approximate, as too little is known of the very variable composition of the products of assimilation. As a rule it will probably be advisable to determine the increase in ash content and deduct it from the increase in dry weight.

2. The tendency of the method to give results which are too high is amply explained by *shrinkage* in area of the experimental half-leaf through loss of turgor during insolation. Shrinkage under insolation is a general phenomenon : it is shown in varying degree by all the leaves examined. Leaves of *Helianthus annuus* often diminish in area by more than 5 per cent. between early morning, when the air is moist, and midday, when the hot sun and dry air favour rapid evaporation. Robust leaves of this plant may show to the eye little sign of flaccidity, even though they are 4 or 5 per cent. less in area than when fully turgid; hence errors from shrinkage cannot be avoided by mere inspection.

The leaves of *Helianthus annuus* are extraordinarily sensitive to changes in the intensity of illumination, and react to them with great rapidity. A leaf was observed to increase or decrease in breadth by nearly 2 per cent. within 10 minutes when the sun passed behind a cloud, or appeared again after having been for a time obscured.

Since this source of error has been practically ignored in all the work hitherto done by the dry weight method, few of the results are trustworthy. Thus the rate of assimilation found by Sachs for attached leaves of *Helianthus annuus*, viz., 9 milligrammes per square decimetre, may easily have been 2 milligrammes in excess of the true rate; while in his similar experiment with *Cucurbita pepo* the whole of the apparent gain may have been illusory.

On the other hand, Sachs' result for detached leaves of *Helianthus annuus*, 16 milligrammes per square decimetre, although so high, is not open to the same

criticism, for he soaked the experimental half-leaves in water at the end of the experiment to make them turgid. Soaking in water does in most cases roughly counteract shrinkage errors, but more accurate means must be adopted for general use.

As an alternative, changes of area may be measured by the planimeter method, or estimated from measurements of the changes in longitudinal dimensions, and a corresponding correction then applied to the increase of dry weight. Errors from shrinkage can also be entirely eliminated by the simpler alternative of marking out a given area on both control and experimental half-leaves at the beginning of the experiment. The *stamping method* has been devised for this purpose: in this method an ink impression of a rectangle is made on each half-leaf with a specially constructed rubber stamp.

3. The other important error to which the dry weight method is liable arises from *asymmetry*, from the fact that equal areas, taken at the same time from the two halves of a leaf, have not accurately the same dry weight. This source of error is inherent in the method and cannot be eliminated: it determines the ultimate limit of the accuracy obtainable.

The error can be diminished: (1) by avoiding outstanding veins, and so reducing the degree of asymmetry shown by individual leaves, and (2) by using a number of leaves for each experiment.

In testing leaves of *Paulownia imperialis*, using only parts without prominent veins, differences of the order of $1\frac{1}{2}$ per cent. were found, whereas portions with the minor veins slightly projecting gave differences *four times as great*.

The reduction of the asymmetry error by the use of a large number of leaves is limited in practice. An improved templet, or the rotating punch, would make possible the simultaneous manipulation of a number of leaves of smooth, firm texture; but the result would have to be corrected for shrinkage, and the estimation of this correction would prove laborious unless it could be made from measurements of a few representative leaves. To deal singly with a large number of leaves would take too long, whatever the method employed.

4. Included in measurements of asymmetry are the experimental errors involved in *measuring the area* and finding the *dry weight* of individual portions of leaf material. Although these are small compared with the error from actual asymmetry, it is nevertheless important to reduce them as much as possible, as they are all cumulative in their effect upon the result.

4A. The important methods of area determination are four in number: Sachs' templet method; Brown and Escombe's planimeter method; and the rotating punch and stamping methods devised in the course of this research.

A test applied to Sachs' *templet method* showed an error which was consider-

ably less than 0·5 per cent. of areas as small as 20 sq. cm.: the other methods are at least as accurate as this.

All the errors of the templet method can be reduced to a minimum by modifying the form of the templet and of the cutting instrument.

By means of the *rotating punch* small discs may be cut from between the veins. Since, however, this method is not a very rapid one it should not be used for leaves like those of *Helianthus annuus,* which are liable to rapid shrinkage.

The *planimeter method* can be used for entire half-leaves, or for pieces cut from between the veins; great care is necessary, however, especially in the latter case, to avoid shrinkage while taking the photographic print.

The stamping method completely eliminates all shrinkage errors; by it the dry weight method is made as simple as possible, but its use is restricted to fairly smooth leaves. With both the templet and stamping methods areas as small as 10 sq. cm. can be safely used; but the disc and planimeter methods are more suitable for very small leaves.

4B. Since dry leaf material is often extremely hygroscopic, it should be dried in a current of dry air and weighed with great care to exclude moisture.

5. To sum up, the main errors involved in the dry-weight method are due to (1) the shrinking of leaves in area during experiment, and (2) their lack of symmetry in respect of dry weight per unit area. Of these errors, those from shrinkage can be eliminated; those from asymmetry, on the other hand, must always be reckoned with. *Asymmetry tests must therefore always form an integral part of dry weight experiments;* especially as any appreciable reduction in *experimental* errors will also appear in these tests.

The relative magnitude of the total error may be reduced by increasing the time of experiment and so distributing it over a larger number of hours.

The real increase of dry weight in a period of 5 *hours or more can be determined with most leaves correct to the nearest milligramme per square decimetre per hour by using appropriate methods.** With shorter experiments this degree of accuracy cannot be reached except with very thin leaves.

Thus, the dry weight method is capable of yielding useful results, for rates of increase greater than 2 milligrammes per square decimetre per hour. It is not to be compared for accuracy with gasometric methods, and can never be expected to afford a means of studying the more refined questions of pure physiology; but the accuracy obtainable will allow of the approximate solution of a number of interesting problems, and will be of service in ecological comparative studies of assimilatory activity.

* This applies strictly only to broad leaves. For narrow linear leaves special methods would have to be devised, and for many very small leaves the half-leaf method is obviously unsuitable.